(54) **Method of manufacturing a stent comprising an anti-angiogenic composition**

Herstellungsmethode eines Stents mit anti-angiogener Zusammensetzung

Procédé de fabrication d'un stent comprenant une composition anti-angiogénique

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Description

Technical Field

5   **[0001]** The present invention relates generally to compositions for treating cancer and other angiogenic-dependent diseases, and more specifically, to compositions comprising anti-angiogenic factors and polymeric carriers, stents which have been coated with such compositions, in particular methods for manufacturing these stents.

Background Of The Invention

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[0002] Cancer is the second leading cause of death in the United States, and accounts for over one fifth of the total mortality. Briefly, cancer is characterized by the uncontrolled division of a population of cells which, most typically, leads to the formation of one or more tumors. Although cancer is generally more readily diagnosed than in the past, many forms, even if detected early, are still incurable.

15   **[0003]** A variety of methods are presently utilized to treat cancer, including for example various surgical procedures. If treated with surgery alone, however, many patients (particularly those with certain types of cancer, such as breast, brain, colon and hepatic cancer) will experience recurrence of the cancer. In addition to surgery, many cancers are also treated with a combination of therapies involving cytotoxic chemotherapeutic drugs (*e.g.*, vincristine, vinblastine, cisplatin, methotrexate, 5-FU, etc.) and/or radiation therapy. One difficulty with this approach, however, is that radiotherapeutic

20   and chemotherapeutic agents are toxic to normal tissues, and often create life threatening side effects. In addition, these approaches often have extremely high failure/remission rates.

[0004] In addition to surgical, chemo and radiation therapies, others have attempted to utilize an individual's own immune system in order to eliminate cancerous cells. For example, some have suggested the use of bacterial or viral components as adjuvants in order to stimulate the immune system to destroy tumor cells: (*See generally* "Principles of

25   Cancer Biotherapy," Oldham (ed.), Raven Press, New York, 1987.) Such agents have generally been useful as adjuvants and as nonspecific stimulants in animal tumor models, but have not as of yet proved to be generally effective in humans.

[0005] Lymphokines have also been utilized in the treatment of cancer. Briefly, lymphokines are secreted by a variety of cells, and generally have an effect on specific cells in the generation of an immune response. Examples of lymphokines include Interleukins (IL)-1, -2, -3, and -4, as well as colony stimulating factors such as G-CSF, GM-CSF, and M-CSF.

30   Recently, one group has utilized IL-2 to stimulate peripheral blood cells in order to expand and produce large quantities of cells which are cytotoxic to tumor cells (Rosenberg et al., N. Engl. J. Med. 313:1485-1492, 1985).

[0006] Others have suggested the use of antibodies in the treatment of cancer. Briefly, antibodies may be developed which recognize certain cell surface antigens that are either unique, or more prevalent on cancer cells compared to normal cells. These antibodies, or "magic bullets," may be utilized either alone or conjugated with a toxin in order to

35   specifically target and kill tumor cells (Dillman, "Antibody Therapy," Principles of Cancer Biotherapy, Oldham (ed.), Raven Press, Ltd., New York, 1987). However, one difficulty is that most monoclonal antibodies are of murine origin, and thus hypersensitivity against the murine antibody may limit its efficacy, particularly after repeated therapies. Common side effects include fever, sweats and chills, skin rashes, arthritis, and nerve palsies.

[0007] One additional difficulty of present methods is that local recurrence and local disease control remains a major

40   challenge in the treatment of malignancy. In particular, a total of 630,000 patients annually (in the U.S.) have localized disease (no evidence of distant metastatic spread) at the time of presentation; this represents 64% of all those patients diagnosed with malignancy (this does not include nonmelanoma skin cancer or carcinoma in *situ*). For the vast majority of these patients, surgical resection of the disease represents the greatest chance for a cure and indeed 428,000 will be cured after the initial treatment - 428,000. Unfortunately, 202,000 (or 32% of all patients with localized disease) will

45   relapse after the initial treatment. Of those who relapse, the number who will relapse due to local recurrence of the disease amounts to 133,000 patients annually (or 21% of all those with localized disease). The number who will relapse due to distant metastases of the disease is 68,000 patients annually (11% of all those with localized disease). Another 102,139 patients annually will die as a direct result of an inability to control the local growth of the disease.

[0008] Nowhere is this problem more evident than in breast cancer, which affects 186,000 women annually in the U.S.

50   and whose mortality rate has remained unchanged for 50 years. Surgical resection of the disease through radical mastectomy, modified radical mastectomy, or lumpectomy remains the mainstay of treatment for this condition. Unfortunately, 39% of those treated with lumpectomy alone will develop a local recurrence of the disease, and surprisingly, so will 25% of those in which the resection margin is found to be clear of tumor histologically. As many as 90% of these local recurrences will occur within 2 cm of the previous excision site.

55   **[0009]** Similarly, in 1991, over 113,000 deaths and 238,600 new cases of liver metastasis were reported in North America alone. The mean survival time for patients with liver metastases is only 6.6 months once liver lesions have developed. Non-surgical treatment for hepatic metastases include systemic chemotherapy, radiation, chemoembolization, hepatic arterial chemotherapy, and intraarterial radiation. However, despite evidence that such treatments can

transiently decrease the size of the hepatic lesions (*e.g.*, systemic chemotherapy and hepatic arterial chemotherapy initially reduces lesions in 15-20%, and 80% of patients, respectively), the lesions invariably reoccur. Surgical resection of liver metastases represents the only possibility for a cure, but such a procedure is possible in only 5% of patients with metastases, and in only 15-20% of patients with primary hepatic cancer.

[0010]　One method that has been attempted for the treatment of tumors with limited success is therapeutic embolization. Briefly, blood vessels which nourish a tumor are deliberately blocked by injection of an embolic material into the vessels. A variety of materials have been attempted in this regard, including autologous substances such as fat, blood clot, and chopped muscle fragments, as well as artificial materials such as wool, cotton, steel balls, plastic or glass beads, tantalum powder, silicone compounds, radioactive particles, sterile absorbable gelatin sponge (Sterispon, Gelfoam), oxidized cellulose (Oxycel), steel coils, alcohol, lyophilized human dura mater (Lyodura), microfibrillar collagen (Avitene), collagen fibrils (Tachotop), polyvinyl alcohol sponge (PVA; Ivalon), Barium-impregnated silicon spheres (Biss) and detachable balloons. The size of liver metastases may be temporarily decreased utilizing such methods, but tumors typically respond by causing the growth of new blood vessels into the tumor.

[0011]　A related problem to tumor formation is the development of cancerous blockages which inhibit the flow of material through body passageways, such as the bile ducts, trachea, esophagus, vasculature and urethra. One device, the stent, has been developed in order to hold open passageways which have been blocked by tumors or other substances. Representative examples of common stents include the Wallstent, Strecker stent, Gianturco stent and the Palmaz stent. The major problem with stents, however, is that they do not prevent the ingrowth of tumor or inflammatory material through the interstices of the stent. If this material reaches the inside of a stent and compromises the stent lumen, it may result in blockage of the body passageway into which it has been inserted. In addition, presence of a stent in the body may induce reactive or inflammatory tissue (*e.g.,* blood vessels, fibroblasts, white blood cells) to enter the stem lumen, resulting in partial or complete closure of the stent.

[0012]　The present invention provides methods of making devices suitable for treating cancers and other angiogenesis-dependent diseases which address the problems associated with the procedures discussed above, and further provides other related advantages.

Summary of the Invention

[0013]　Briefly stated, the present invention as claimed relates to methods of making devices which utilize anti-angiogenic compositions for the treatment of cancer and other angiogenesis-dependent diseases.. Within one aspect, compositions are provided (hereinafter referred to as "anti-angiogenic compositions") comprising (a) an anti-angiogenic factor and (b) a polymeric carrier. A wide variety of molecules may be utilized within the scope of the present invention as anti-angiogenic factors, in particular taxol, taxol analogues and taxol derivatives. Similarly, a wide variety of polymeric carriers may be utilized, representative examples of which include poly(ethylene-vinyl acetate) crosslinked with 40% vinyl acetate, poly (lactic-co-glycolic acid), polycaprolactone polylactic acid, copolymers of poly(ethylene-vinyl acetate) crosslinked with 40% vinyl acetate and polylactic acid, and copolymers of polylactic acid and polycaprolactone. Within one embodiment of the invention, the composition has an average size of 15 to 200 μm.

[0014]　Stents are provided comprising a generally tubular structure, the surface being coated with one or more anti-angiogenic compositions. Methods are provided for expanding the lumen of a body passageway, comprising inserting a stent into the passageway, the stent having a generally tubular structure, the surface of the structure being coated with an anti-angiogenic composition as described above, such that the passageway is expanded. Methods are provided for eliminating biliary obstructions, comprising inserting a biliary stent into a biliary passageway; for eliminating urethral obstructions, comprising inserting a urethral stent into a urethra; for eliminating esophageal obstructions, comprising inserting an esophageal stent into an esophagus; and for eliminating tracheal/bronchial obstructions, comprising inserting a tracheal/bronchial stent into the trachea or bronchi. In each of these embodiments, the stent has a generally tubular structure, the surface of which is coated with an anti-angiogenic composition as described above.

[0015]　Methods are provided for expanding the lumen of a body passageway, comprising inserting a stent into the passageway, the stent having a generally tubular structure, the surface of the structure being coated with a composition comprising taxol, such that the passageway is expanded. Methods are provided for eliminating biliary obstructions, comprising inserting a biliary stent into a biliary passageway; for eliminating urethral obstructions, comprising inserting a urethral stent into a urethra; for eliminating esophageal obstructions, comprising inserting an esophageal stent into an esophagus; and for eliminating tracheal/broncbial obstructions, comprising inserting a tracheal/bronchial stent into the trachea or bronchi. Within each of these embodiments the stent has a generally tubular structure, the surface of the structure being coated with a composition comprising taxol.

[0016]　These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings. In addition, various references are set forth below which describe in more detail certain procedures or compositions .

Brief Description of the Drawings

[0017]

Figure 1A is a photograph which shows a shell-less egg culture on day 6. Figure 1B is a digitized computer-displayed image taken with a stereomicroscope of living, unstained capillaries (1040x). Figure 1C is a corrosion casting which shows CAM microvasculature that are fed by larger, underlying vessels (arrows; 1300x). Figure 1D depicts a 0,5 mm thick plastic section cut transversely through the CAM, and recorded at the light microscope level. This photograph shows the composition of the CAM, including an outer double-layered ectoderm (Ec), a mesoderm (M) containing capillaries (arrows) and scattered adventitila cells, and a single layered endoderm (En) (400x). Figure 1E is a photograph at the electron microscope level (3500x) wherein typical capillary structure is presented showing thin-walled endothelial cells, (arrowheads) and an associated pericyte.

Figures 2A, 2B, 2C and 2D are a series of digitized images of four different, unstained CAMs taken after a 48 hour exposure to taxol.

Figures 3A, 3B and 3C are a series of photographs of 0.5 mm thick plastic sections transversely cut through a taxol-treated CAM at three different locations within the avascular zone.

Figures 4A, 4B and 4C are series of electron micrographs which were taken from locations similar to that of Figures 3A, 3B and 3C (respectively) above.

Figure 5 is a bar graph which depicts the size distribution of microspheres by number (5% ELVAX with 10 mg sodium suramin into 5% PVA).

Figure 6 is a bar graph which depicts the size distribution of microspheres by weight (5% ELVAX with 10 mg sodium suramin into 5% PVA).

Figure 7 is a line graph which depicts the weight of encapsulation of Sodium Suramin in 1 ml of 5% ELVAX.

Figure 8 is a line graph which depicts the percent of encapsulation of Sodium Suramin in ELVAX.

Figure 9 is a bar graph which depicts the size distribution of 5% ELVAX microspheres containing 10 mg sodium suramin made in 5% PVA containing 10% NaCl.

Figure 10 is a bar graph which depicts the size distribution by weight of 5% PLL microspheres containing 10 mg sodium suramin made in 5% PVA containing 10% NaCl.

Figure 11 is a bar graph which depicts the size distribution by number of 5% PLL microspheres containing 10 mg sodium suramin made in 5% PVA containing 10% NaCl.

Figure 12 is a line graph which depicts the time course of sodium suramin release.

Figure 13 is an illustration of a representative embodiment of hepatic tumor embolization.

Figure 14 is an illustration of the insertion of a representative stent coated with an anti-angiogenic composition of the present invention.

Figure 15A is a graph which shows the effect of the EVA:PLA polymer blend ratio upon aggregation of microspheres. Figure 15B is a scanning electron micrograph which shows the size of "small" microspheres. Figure 15C is a scanning electron micrograph which shows the size of "large" microspheres. Figure 15D is a graph which depicts the time course of *in vitro* taxol release from 0.6% w/v taxol-loaded 50:50 EVA:PLA polymers blend microspheres into phosphate buffered saline (pH 7.4) at 37°C Open circles are "small" sized microspheres, and closed circles are "large" sized microspheres. Figure 15E is a photograph of a CAM which shows the results of taxol release by microspheres ("MS"). Figure 15F is a photograph similar to that of 15E at increased magnification.

Figure 16 is a graph which shows release rate profiles from polycaprolactone microspheres containing 1%, 2%, 5% or 10% taxol into phosphate buffered saline at 37°C Figure 16B is a photograph which shows a CAM treated with control microspheres. Figure 16C is a photograph which shows a CAM treated with 5% taxol loaded microspheres.

Figures 17A and 17B, respectively, are two graphs which show the release of taxol from EVA films, and the percent taxol remaining in those same films over time. Figure 17C is a graph which shows the swelling of EVA/F127 films with no taxol over time. Figure 17D is a graph which shows the swelling of EVA/Span 80 films with no taxol over time. Figure 17E is a graph which depicts a stress vs. strain curve for various EVA/F127 blends.

Figures 18A and 18B are two graphs which show the melting point of PCL/MePEG polymer blends as a function of % MePEG in the formulation (18A), and the percent increase in time needed for PCL paste at 60°C to begin to solidify as a function of the amount of MePEG in the formulation (18B). Figure 18C is a graph which depicts the brittleness of varying PCL/MePEG polymer blends.. Figure 18D is a graph which shows the percent weight change over time for polymer blends of various MePEG concentrations. Figure 18E is a graph which depicts the rate of taxol release over time from various polymer blends loaded with 1% taxol. Figures 18F and 18G are graphs which depict the effect of varying quantities of taxol on the total amount of taxol released from a 20%MePEG/PCL blend. Figure 18H is a graph which depicts the effect of MePEG on the tensile strength of a MePEG/PCL polymer.

Figure 19A is a photograph which shows control (unloaded) thermopaste on a CAM. Figure 19B is a photograph of 20% taxol-loaded thermopaste on a CAM.

Figures 20A and 20B are two photographs of a CAM having a tumor treated with control (unloaded) thermopaste. Figures 20C and 20D are two photographs of a CAM having a tumor treated with taxol-loaded thermopaste.

Figure 21A is a graph which shows the effect of taxol/PCL on tumor growth. Figures 21B and 21C are two photographs which show the effect of control, 10%, and 20% taxol-loaded thermopaste on tumor growth.

Detailed Description of the Invention

[0018] As noted above, the present invention provides methods of making devices comprising compositions which utilize anti-angiogenic factors. The method which may be readily utilized to determine the anti-angiogenic activity of a given factor is the chick chorioallantoic membrane ("CAM") assay. Briefly, as described in more detail below in Reference Examples 1A and 1C, a portion of the shell from a freshly fertilized chicken egg is removed, and a methyl cellulose disk containing a sample of the anti-angiogenic factor to be tested is placed on the membrane. After several days (*e.g.*, 48 hours), inhibition of vascular growth by the sample to be tested may be readily determined by visualization of the chick chorioallantoic membrane in the region surrounding the methyl cellulose disk. Inhibition of vascular growth may also be determined quantitatively, for example, by determining the number and size of blood vessels surrounding the methyl cellulose disk, as compared to a control methyl cellulose disk. Particularly preferred anti-angiogenic factors suitable for use within the present invention completely inhibit the formation of new blood vessels in the assay described above.

[0019] A variety of assays may also be utilized to determine the efficacy of anti-angiogenic factors in *vivo,* including for example, mouse models which have been developed for this purpose (see Roberston et al., Cancer. Res. 51: 1339-1344, 1991). In addition, a variety of representative *in vivo* assays relating to various aspects of the inventions described herein have been described in more detail below in Reference Examples 4, 5 and 15.

[0020] As noted above, the present disclosure provides compositions comprising an anti-angiogenic factor and a polymeric carrier. Anti-angiogenic factors which may be utilized within the context of the present invention include taxol This will be discussed in more detail below.

[0021] Taxol is a highly derivatized diterpenoid (Wani et al., J. Am. Chem. Soc. 93:2325, 1971) which has been obtained from the harvested and dried bark of *Tarus brevifolia* (Pacific Yew) and *Taxomyces Andreanae* and *Endophytic Fungus* of the Pacific Yew. (Stierle et al., Science 60:214-216, 1993). Generally, taxol acts to stabilize microtubular structures by binding tubulin to form abnormal mitotic spindles. "Taxol" (which should be understood herein to include analogues and derivatives of taxol such as, for example, baccatin and taxotere) may be readily prepared utilizing techniques known to those skilled in the art (see also WO 94/07882, WO 94/01881, WO 94/07880, WO 94/07876, WO 93/23555, WO 93/10076, U.S. Patent Nos. 5,294,637, 5,283,353, 5,279,949, 5,274,137, 5,202,448, 5,200,534, 5,229,526, and EP 590267) or obtained from a variety of commercial sources, including for example, Sigma Chemical Co., St. Louis, Missouri (T7402 - from *Taxus brevifolia*).

[0022] Anti-angiogenic compositions used in the present invention may additionally comprise a wide variety of compounds in addition to the anti-angiogenic factor and polymeric carrier. For example, anti-angiogenic compositions of the present invention may also, within certain embodiments of the invention, also comprise one or more antibiotics, anti-inflamatories, antiviral agents, anti-fungal agents and/or anti-protozoal agents. Representative examples of antibiotics included within the compositions described herein include: penicillins; cephalosporins such as cefadroxil, cefazolin cefaclor; aminoglycosides such as gentamycin and tobramycin; sulfonamides such as sulfamethoxazole; and metronidazole. Representative examples of antiinflammatories include: steroids such as prednisone, prednisolone, hydrocortisone, adrenocorticotropic hormone, and sulfasalazine; and non-steroidal anti-inflammatory drugs ("NSAIDS") such as aspirin, ibuprofen, naproxen, fenoporfen, indomethacin, and phenylbutazone. Representative examples of antiviral agents include acyclovir, ganciclovir, zidovudine. Representative examples of antifungal agents include: nystatin, ketoconazole, griseofulvin, flucytosine, miconazole, clotrimazole. Representative examples of antiprotozoal agents include: pentamidine isethionate, quinine, chloroquine, and mefloquine

[0023] Anti-angiogenic compositions used in the present invention may also contain one or more hormones such as thyroid hormone, estrogen, progesterone, cortisone and/or growth hormone, other biologically active molecules such as insulin, as well as T_{H1} (*e.g.*, Interleukins -2, -12, and -15, gamma interferon or T_{H2} (*e.g.*, Interleukins -4 and -10) cytokines.

[0024] Anti-angiogenic compositions used in the present invention may also comprise additional ingredients such as surfactants (either hydrophilic or hydrophobic, see Example 13), anti-neoplastic or chemotherapeutic agents (*e.g.*, 5-fluorouracil, vinblastine, doxyrubicin, adriamycin, or tamocifen), radioactive 5 agents (*e.g.*, Cu-64, Ga-67, Ga-68, Zr-89, Ru-97, Tc-99m, Rh-105, Pd-109, In-111, I-123, I-I25, I-131, Re-186, Re-188, Au-198, Au-199, Pb-203, At-211, Pb-212 and Bi-212) or toxins (*e.g.*, ricin, abrin, diptheria toxin, cholera toxin, gelonin, pokeweed antiviral protein, tritin, Shigella toxin, and Pseudomonas exotoxin A).

[0025] As noted above, anti-angiogenic compositions used in the present invention comprise an anti-angiogenic factor and a polymeric carrier. In addition to the wide array of anti-angiogenic factors and other compounds discussed above, anti-angiogenic compositions used in the present invention may include a wide variety of polymeric carriers, including

for example both biodegradable and non-biodegradable compositions. Representative examples of biodegradable compositions include albumin, gelatin, starch, cellulose, destrans, polysaccharides, fibrinogen, poly (d,l lactide), poly (d,1-lactide-coglycolide), poly (glycolide), poly (hydroxybutyrate), poly (alkylcarbonate) and poly (orthoesters) (*see generally,* Illum, L., Davids, S.S. (eds.) "Polymers in controlled Drug Delivery" Wright, Bristol, 1987; Arshady, J. Controlled Release 77:1-22, 1991; Pitt, Int. J. Phar. 59:173-196, 1990; Holland et al., J. Controlled Release 4:155-0180, 1986). Representative examples of nondegradable polymers include EVA copolymers, silicone rubber and poly (methylmethacrylate). Particularly preferred polymeric carriers include EVA copolymer (*e.g.,* ELVAX 40, poly(ethylene-vinyl acetate) crosslinked with 40% vinyl acetate; DuPont), poly(lactic-co-glycolic acid), polycaprolactone, polylactic acid, copolymers of poly(ethylene-vinyl acetate) crosslinked with 40% vinyl acetate and polylactic acid, and copolymers of polylactic acid and polycaprolactone.

[0026]   Polymeric carriers may be fashioned in a variety of forms, including for example, as nanospheres or microspheres, rod-shaped devices, pellets, slabs, or capsules (*see, e.g.,* Goodell et al., Am. J. Hosp. Pharm. 43:1454-1461, 1986; Langer et al., "Controlled release of macromolecules from polymers", in Biomedical polymers, Polymeric materials and pharmaceuticals for biomedical use, Goldberg, E.P., Nakagim, A. (eds.) Academic Press, pp. 113-137, 1980; Rhine et al., J. Pharm. Sci. 69:265-270, 1980; Brown et al., J. Pharm. Sci. 72:1181-1185, 1983; and Bawa et al., J. Controlled Release 1:259-267, 1985).

[0027]   Preferable, anti-angiogenic compositions used in the present invention are fashioned in a manner appropriate to the intended use. Within preferred aspects of the present invention, the anti-angiogenic composition should be biocompatible, and release one or more anti-angiogenic factors over a period of several weeks to months. In addition, anti-angiogenic compositions used in the present invention should preferably be stable for several months and capable of being produced and maintained under sterile conditions.

[0028]   Within certain aspects of the present invention, anti-angiogenic compositions may be fashioned in any size ranging from nanospheres to microspheres (*e.g.,* from 0.1 μm to 500 μm), depending upon the particular use. For example, when used for the purpose of tumor embolization (as discussed below), it is generally preferable to fashion the anti-angiogenic composition in microspheres of between 15 and 500 μm, preferably between 15 and 200 μm, and most preferably, between 25 and 150 μm. Such nanoparticles may also be readily applied as a "spray", which solidifies into a film or coating. Nanoparticles (also termed "nanospheres") may be prepared in a wide array of sizes, including for example, from 0.1 μm to 3 μm, from 10 μm to 30 μm, and from 30 μm to 100 μm (see Reference Example 6).

[0029]   Anti-angiogenic compositions use in the present invention may also be prepare in a variety of "paste" or gel forms. For example, within one embodiment of the invention, anti-angiogenic compositions are provided which are liquid at one temperature (*e.g.,* temperature greater than 37°C, such as 40°C, 45°C, 50°C, 55°C or 60°C), and solid or semi-solid at another temperature (*e.g.,* ambient body temperature, or any temperature lower than 37°C). Such "thermopastes" may be readily made given the disclosure provided herein (*see, e.g.,* Reference Examples 8 and 12).

[0030]   Within yet other aspects of the invention, the anti-angiogenic compositions used in the present invention may be formed as a film. Preferably, such films are generally less than 5, 4, 3, 2, or 1, mm thick, more preferably less than 0.75 mm or 0.5 mm thick, and most preferably less than 500 μm to 100 μm thick. Such films are preferably flexible with a good tensile strength (*e.g.,* greater than 50, preferably greater than 100, and more preferably greater than 150 or 200 N/cm^2), good adhesive properties (*i.e.,* readily adheres to moist or wet surfaces), and has controlled permeability. Representative examples of such films are set forth below in the Examples (*see e.g.,* Reference Example 11).

[0031]   Representative examples of the incorporation of anti-angiogenic factors such as into a polymeric carriers are described in more detail below in Reference Examples , 3, 6 and 8 to 13, and Example 1.

USE OF ANTI-ANGIOGENIC COMPOSITIONS AS COATINGS FOR STENTS

[0032]   As noted above, the present invention also provides methods at making stents, comprising a generally tubular structure (which includes for example, spiral shapes), the surface of which is coated with a composition as described above. Briefly, a stent is a scaffolding, usually cylindrical in shape, that may be inserted into a body passageway (*e.g.,* bile ducts), which has been narrowed by a disease process (*e.g.,* ingrowth by a tumor) in order to prevent closure or reclosure of the passageway. Stents act by physically holding open the walls of the body passage into which they are inserted.

[0033]   A variety of stents may be utilized within the context of the present invention, including for example, esophageal stents, vascular stents, biliary stents, pancreatic stents, ureteric and urethral stents, lacrimal stents, eustachian tube stents, fallopian tube stents, and tracheal/bronchial stents.

[0034]   Stents may be readily obtained from commercial sources, or constructed in accordance with well known techniques. Representative examples of stents include those described in U.S. Patent No. 4,776,337, entitled "Expandable Intraluminal Graft, and Method and Apparatus for Implanting and Expandable Intraluminal Graft", U.S. Patent No. 5,176,626, entitled "Indwelling Stent"; U.S. Patent No. 5,147,370 entitled "Nitinol Stent for Hollow Body Conduits", U.S. Patent No. 5,064,433 entitled "Self-Expanding Prosthesis Having Stable Axial Length", U.S. Patent No. 5,052,998 entitled

"Indwelling Stent and Method of Use", and U.S. Patent No. 5,041,126 entitled "Endovascular Stent and Delivery System .

[0035]   Stents may be coated with anti-angiogenic compositions or anti-angiogenic factors by the method of the present invention by directly affixing to the stent an anti-angiogenic composition (*e.g.*, by either spraying the stent with a polymer/ drug film, or by dipping the stent into a polymer/drug solution) Within preferred embodiments the composition should firmly adhere to the stent during storage and at the time of insertion, and should not be dislodged from the stent when the diameter is expanded from its collapsed size to its full expansion size. The anti-angiogenic composition should also preferably not degrade during storage, prior to insertion, or when warmed to body temperature after expansion inside the body. In addition, it should preferably coat the stent smoothly and evenly, with a uniform distribution of angiogenesis inhibitor, while not changing the stent contour. Within preferred embodiments of the invention, the anti-angiogenic com- position should provide a uniform, predictable, prolonged release of the anti-angiogenic factor into the tissue surrounding the stent once it has been deployed. For vascular stents, in addition to the above properties, the composition should not render the stent thrombogenic (causing blood clots to form), or cause significant turbulence in blood flow (more than the stent itself would be expected to cause if it was uncoated).

[0036]   Methods are provided for expanding the lumen of a body passageway, comprising inserting a stent into the passageway, the stent having a generally tubular structure, the surface of the structure being coated with an anti- angiogenic composition (or, an anti-angiogenic factor alone), such that the passageway is expanded. A variety of em- bodiments are described below wherein the lumen of a body passageway is expanded in order to eliminate a biliary, esophogeal, tracheal/bionchial, urethral or vascular obstruction. In addition, a representative example is described in more detail below in Reference Example 5.

[0037]   Generally, stents are inserted in a similar fashion regardless of the site or the disease being treated. Briefly, a preinsertion examination, usually a diagnostic imaging procedure, endoscopy, or direct visualization at the time of surgery, is generally first performed in order to determine the appropriate positioning for stent insertion. A guidewire is then advanced through the lesion or proposed site of insertion, and over this is passed a delivery catheter which allows a stent in its collapsed form to be inserted. Typically, stents are capable of being compressed, so that they can be inserted through tiny cavities via small catheters, and then expanded to a larger diameter once they are at the desired location. Once expanded, the stent physically forces the walls of the passageway apart and holds it open. As such, they are capable of insertion via a small opening, and yet are still able to hold open a large diameter cavity or passageway. The stent may be self-expanding (*e.g.*, the Wallstent and Gianturco stents), balloon expandable (*e.g.*, the Palmaz stent and Strecker stent), or implanted by a change in temperature (*e.g.*, the Nitinol stent).

[0038]   Stents are typically maneuvered into place under radiologic or direct visual control, taking particular care to place the stent precisely across the narrowing in the organ being treated. The delivery catheter is then removed, leaving the stent standing on its own as a scaffold. A post insertion examination, usually an x-ray, is often utilized to confirm appropriate positioning.

[0039]   Methods are provided for eliminating biliary obstructions, comprising inserting a biliary stent into a biliary pas- sageway, the stent having a generally tubular structure, the surface of the structure being coated with a composition as described above, such that the biliary obstruction is eliminated. Briefly, rumor overgrowth of the common bile duct results in progressive cholestatic jaundice which is incompatible with life.. Generally, the biliary system which drains bile from the liver into the duodenum is most often obstructed by (1) a tumor composed of bile duct cells (cholangiocarcinoma), (2) a tumor which invades the bile duct (*e.g.*, pancreatic carcinoma), or (3) a tumor which exerts extrinsic pressure and compresses the bile duct (*e.g*, enlarged lymph nodes).

[0040]   Both primary biliary tumors, as well as other tumors which cause compression of the biliary tree may be treated utilizing the stents described herein. One example of primary biliary tumors are adenocarcinomas (which are also called Klatskin tumors when found at the bifurcation of the common hepatic duct). These tumors are also referred to as biliary carcinomas, choledocholangiocarcinomas, or adenocarcinomas of the biliary system. Benign tumors which affect the bile duct (*e.g.*, adenoma of the biliary system), and, in rare cases, squamous cell carcinoma of the bile duct and aden- ocarcinomas of the gallbladder, may also cause compression of the biliary tree, and therefore, result in biliary obstruction.

[0041]   Compression of the biliary tree is most commonly due to tumors of the liver and pancreas which compress and therefore obstruct the ducts. Most of the tumors from the pancreas arise from cells of the pancreatic ducts. This is a highly fatal form of cancer (5% of all cancer deaths; 26,000 new cases per year in the U.S.) with an average of 6 months survival and a 1 year survival rate of only 10%. When these tumors are located in the head of the pancreas they frequently cause biliary obstruction, and this detracts significantly from the quality of life of the patient. While all types of pancreatic tumors are generally referred to as "carcinoma of the pancreas," there are histologic subtypes including: adenocarcinoma, adenosqusmous carcinoma, cystadeno-carcinoma, and acinar cell carcinoma. Hepatic tumors, as discussed above, may also cause compression of the biliary tree, and therefore cause obstruction of the biliary ducts.

[0042]   A biliary stent may first inserted into a biliary passageway in one of several ways: from the top end by inserting a needle through the abdominal wall and through the liver (a percutaneous transhepatic cholangiogram or "PTC"); from the bottom end by cannulating the bile duct through an endoscope inserted through the mouth, stomach, or duodenum (an endoscopic retrograde cholangiogram or "ERCP"); or by direct incision during a surgical procedure.. A preinsertion

examination, PTC, ERCP, or direct visualization at the time of surgery should generally be performed to determine the appropriate position for stent insertion. A guidewire is then advanced through the lesion, and over this a delivery catheter is passed to allow the stent to be inserted in its collapsed form. If the diagnostic exam was a PTC, the guidewire and delivery catheter will be inserted via the abdominal wall, while if the original exam was an ERCP the stent will be placed via the mouth. The stent is then positioned under radiologic, endoscopic, or direct visual control taking particular care to place it precisely accross the narrowing in the bile duct. The delivery catheter will be removed leaving the stent standing as a scaffolding which holds the bile duct open. A further cholangiogram will be performed to document that the stent is appropriately positioned.

[0043] Methods are provided for eliminating esophageal obstructions, comprising inserting an esophageal stent into an esophagus, the stent having a generally tubular structure, the surface of the structure being coated with an anti-angiogenic composition as described above, such that the esophageal obstruction is eliminated. Briefly, the esophagus is the hollow tube which transports food and liquids from the mouth to the stomach. Cancer of the esophagus or invasion by cancer arising in adjacent organs (*e.g.*, cancer of the stomach or lung) results in the inability to swallow food or saliva. Within this embodiment, a preinsertion examination, usually a barium swallow or endoscopy should generally be performed in order to determine the appropriate position for stent insertion. A catheter or endoscope may then be positioned through the mouth, and a guidewire is advanced through the blockage. A stent delivery catheter is passed over the guidewire under radiologic or endoscopic control, and a stent is placed precisely across the narrowing in the esophagus. A post insertion examination, usually a barium swallow x-ray, may be utilized to confirm appropriate positioning.

[0044] Methods are provided for eliminating tracheal/bronchial obstructions, comprising inserting a tracheral/bronchial stent into the trachea or bronchi, the stent having a generally tubular structure, the surface of which is coated with an anti-angiogenic composition as described above, such that the tracheal/bronchial obstruction is eliminated. Briefly, the trachea and bronchi are tubes which carry air from the mouth and nose to the lungs.. Blockage of the trachea by cancer, invasion by cancer arising in adjacent organs (*e.g.*, cancer of the lung), or collapse of the trachea or bronchi due to chondromalacia (weakening of the cartilage rings) results in inability to breathe. Within this embodiment, preinsertion examination, usually an endoscopy, should generally be performed in order to determine the appropriate position for stent insertion. A catheter or endoscope is then positioned through the mouth, and a guidewire advanced through the blockage. A delivery catheter is then passed over the guidewire in order to allow a collapsed stem to be inserted. The stent is placed under radiologic or endoscopic control in order to place it precisely across the narrowing. The delivery catheter may then be removed leaving the stent standing as a scaffold on its own. A post insertion examination, usually a bronchoscopy, may be utilized to confirm appropriate positioning.

[0045] Methods are provided for eliminating urethral obstructions, comprising inserting a urethral stent into a urethra, the stent having a generally tubular structure, the surface of the structure being coated with an anti-angiogenic composition as described above, such that the urethral obstruction is eliminated. Briefly, the urethra is the tube which drains the bladder through the penis. Extrinsic narrowing of the urethra as it passes through the prostate, due to hypertrophy of the prostate, occurs in virtually every man over the age of 60 and causes progressive difficulty with urination. Within this embodiment, a preinsertion examination, usually an endoscopy or urethrogram should generally first be performed in order to determine the appropriate position for stent insertion, which is above the external urinary sphincter at the lower end, and close to flush with the bladder neck at the upper end. An endoscope or catheter is then positioned through the penile opening and a guidewire advanced into the bladder. A delivery catheter is then passed over the guidewire in order to allow stent insertion. The delivery catheter is then removed, and the stent expanded place. A post insertion examination, usually endoscopy or retrograde urethrogram, may be utilized to confirm appropriate position.

[0046] Methods are provided for eliminating vascular obstructions, comprising inserting a vascular stent into a blood vessel, the stent having a generally tubular structure, the surface of the structure being coated with an anti-angiogenic composition as described above, such that the vascular obstruction is eliminated. Briefly, stents may be placed in a wide array of blood vessels, both arteries and veins, to prevent recurrent stenosis at the site of failed angioplasties, to treat narrowings that would likely fail if treated with angioplasty, and to treat post surgical narrowings (*e.g.*, dialysis graft stenosis). Representative examples of suitable sites include the iliac, renal, and coronary arteries, the superior vena cava, and in dialysis grafts. Within one embodiment, angiography is first performed in order to localize the site for placement of the stent. This is typically accomplished by injecting radiopaque contrast through a catheter inserted into an artery or vein as an x-ray is taken. A catheter may then be inserted either percutaneously or by surgery into the femoral artery, brachial artery, femoral vein, or brachial vein, and advanced into the appropriate blood vessel by steering it through the vascular system under fluoroscopic guidance. A stent may then be positioned across the vascular stenosis. A post insertion angiogram may also be utilized in order to confirm appropriate positioning.

[0047] The following example are offered by way of illustration, and not by way of limitation.

Refernece <u>EXAMPLE 1</u>

ANALYSIS OF VARIOUS AGENTS FOR ANTI-ANGIOGENIC ACTIVITY

A. <u>Chick-Chorioallantoic Membrane ("Cam) Assays</u>

[0048]   Fertilized, domestic chick embryos were incubated for 3 days prior to shell-less culturing. In this procedure, the egg contents were emptied by removing the shell located around the air space. The interior shell membrane was then severed and the opposite end of the shell was perforated to allow the contents of the egg to gently slide out from the blunted end. The egg contents were emptied into round-bottom sterilized glass bowls and covered with petri dish covers. These were then placed into an incubator at 90% relative humidity and 3% CO_2 and incubated for 3 days.

[0049]   Taxol (Sigma, St. Louis, MI) was mixed at concentrations of 1, 5, 10, 30mg per 10ml aliquot of 0.5% aqueous methylcellulose. Since taxol is insoluble in water, glass beads were used to produce fine particles. Ten microliter aliquots of this solution were dried on parafilm for 1 hour forming disks 2mm in diameter. The dried disks containing taxol were then carefully placed at the growing edge of each CAM at day 6 of incubation. Controls were obtained by placing taxol-free methylcellulose disks on the CAMs over the same time course. After a 2 day exposure (day 8 of incubation) the vasculature was examined with the aid of a stereomicroscope. Liposyn II, a white opaque solution, was injected into the CAM to increase the visibility of the vascular details. The vasculature of unstained. living embryos were imaged using a Zeiss stereomicroscope which was interfaced with a video camera (Dage-MTI Inc., Michigan City, IN). These video signals were then displayed at 160 times magnification and captured using an image analysis system (Vidas, Kontron; Etching, Germany). Image negatives were then made on a graphics recorder (Model 3000; Matrix Instruments, Orange-burg, NY).

[0050]   The membranes of the 8 day-old shell-less embryo were flooded with 2% glutaraldehyde in 0.1M Na cacodylate buffer; additional fixative was injected under the CAM. After 10 minutes *in situ,* the CAM was removed and placed into fresh fixative for 2 hours at room temperature. The tissue was then washed overnight in cacodylate buffer containing 6% sucrose. The areas of interest were postfixed in 1% osmium tetroxide for 15 hours at 4°C. The tissues were then dehydrated in a graded series of ethanols, solvent exchanged with propylene oxide, and embedded in Spurr resin. Thin sections were cut with a diamond knife, placed on copper grids, stained, and examined in a Joel 1200EX electron microscope. Similarly, 0.5 mm sections were cut and stained with toluene blue for light microscopy.

[0051]   At day 11 of development, chick embryos were used for the corrosion casting technique. Mercox resin (Ted Pella, Inc., Redding, CA) was injected into the CAM vasculature using a 30-gauge hypodermic needle. The casting material consisted of 2.5 grams of Mercox CL-2B polymer and 0.05 grams of catalyst (55% benzoyl peroxide) having a 5 minute polymerization time. After injection, the plastic was allowed to sit *in situ* for an hour at room temperature and then overnight in an oven at 65°C. The CAM was then placed in 50% aqueous solution of sodium hydroxide to digest all organic components. The plastic casts were washed extensively in distilled water, air-dried, coated with gold/palladium, and viewed with the Philips 501B scanning electron microscope.

[0052]   Results of the above experiments are shown in Figures 1-4. Briefly, the general features of the normal chick shell-less egg culture are shown in Figure 1A. At day 6 of incubation, the embryo is centrally positioned to a radially expanding network of blood vessels; the CAM develops adjacent to the embryo. These growing vessels lie close to the surface and are readily visible making this system an idealized model for the study of angiogenesis. Living, unstained capillary networks of the CAM can be imaged noninvasively with a stereomicroscope. Figure 1B illustrates such a vascular area in which the cellular blood elements within capillaries were recorded with the use of a video/computer interface. The 3-dimensional architecture of such CAM capillary networks is shown by the corrosion casting method and viewed in the scanning electron microscope (Figure 1C). These castings revealed underlying vessels which project toward the CAM surface where they form a single layer of anastomotic capillaries.

[0053]   Transverse sections through the CAM show an outer ectoderm consisting of a double cell layer, a broader mesodermal layer containing capillaries which lie subjacent to the ectoderm, adventitial cells, and an inner, single endodermal cell layer (Figure 1D). At the electron microscopic level, the typical structural details of the CAM capillaries are demonstrated. Typically, these vessels lie in close association with the inner cell layer of ectoderm (Figure 1E)

[0054]   After 48 hours exposure to taxol at concentrations of 1, 5, 10, or 30 mg, each CAM was examined under living conditions with a stereomicroscope equipped with a video/computer interface in order to evaluate the effects on angio-genesis. This imaging setup was used at a magnification of 160 times which permitted the direct visualization of blood cells within the capillaries; thereby blood flow in areas of interest could be easily assessed and recorded. For this study, the inhibition of angiogenesis was defined as an area of the CAM devoid of a capillary network ranging from 2-6 mm in diameter. Areas of inhibition lacked vascular blood flow and thus were only observed under experimental conditions of methylcellulose containing taxol; under control conditions of disks lacking, taxol there was no effect on the developing capillary system. The dose-dependent, experimental data of the effects of taxol at different concentrations are shown in Table II.

TABLE II

Angiogenic Inhibition by Taxol		
Taxol Concentration μg	Embroys Evaluated (Positive/Total)	% Inhibition
30	31/31	100
10	16/21	76
5	18/25	72
1	6/15	40
Control	0/30	0

[0055]   Typical taxol-treated CAMs (Figures 2A and 2B) are shown with the transparent methylcellulose disk centrally positioned over the avascular zone measuring 6 mm in diameter. At a slightly higher magnification, the periphery of such avascular zones is clearly evident (Figure 2C); the surrounding functional vessels were often redirected away from the source of taxol (Figures 2C and 2D). Such angular redirecting of blood flow was never observed under normal conditions. Another feature of the effects of taxol was the formation of blood islands within the avascular zone representing the aggregation of blood cells.

[0056]   The associated morphological alterations of the taxol-treated CAM are readily apparent at both the light and electron microscopic levels. For the convenience of presentation, three distinct phases of general transition from the normal to the avascular state are shown. Near the periphery of the avascular zone the CAM is hallmarked by an abundance of mitotic cells within all three germ layers (Figures 3A and 4A). This enhanced mitotic division was also a consistent observation for capillary endothelial cells. However, the endothelial cells remained junctionally intact with no extravasation of blood cells. With further degradation, the CAM is characterized by the breakdown and dissolution of capillaries (Figures 3B and 4B). The presumptive endothelial cells, typically arrested in mitosis, still maintain a close spatial relationship with blood cells and lie subjacent to the ectoderm; however, these cells are not junctionally linked. The most central portion of the avascular zone was characterized by a thickened ectodermal and endodermal layer (Figures 3C and 4C). Although these layers were thickened, the cellular junctions remained intact and the layers maintained their structural characteristics. Within the mesoderm, scattered mitotically arrested cells were abundant; these cells did not exhibit the endothelial cell polarization observed in the former phase. Also, throughout this avascular region, degenerating cells were common as noted by the electron dense vacuoles and cellular debris (Figure 4C).

[0057]   In summary, this study demonstrated that 48 hours after taxol application to the CAM, angiogenesis was inhibited. The blood vessel inhibition formed an avascular zone which was represented by three transitional phases of taxol's effect. The central, most affected area of the avascular zone contained disrupted capillaries with extravasated red blood cells; this indicated that intercellular junctions between endothelial cells were absent. The cells of the endoderm and ectoderm maintained their intercellular junctions and therefore these germ layers remained intact; however, they were slightly thickened. As the normal vascular area was approached, the blood vessels retained their junctional complexes and therefore also remained intact. At the periphery of the taxol-treated zone, further blood vessel growth was inhibited which was evident by the typical redirecting or "elbowing" effect of the blood vessels (Figure 24D).

[0058]   Taxol-treated avascular zones also revealed an abundance of cells arrested in mitosis in all three germ layers of the CAM; this was unique to taxol since no previous study has illustrated such an event. By being arrested in mitosis, endothelial cells could not undergo their normal metabolic functions involved in angiogenesis. In comparison, the avascular zone formed by suramin and cortisone acetate do not produce mitotically arrested cells in the CAM; they only prevented further blood vessel growth into the treated area. Therefore, even though agents are anti-angiogenic, there are many points in which the angiogenesis process may be targetted.

[0059]   We also observed the effects of taxol over the 48 hour duration and noticed that inhibition of angiogenesis occurs as early as 9 hours after application. Histological sections revealed a similar morphology as seen in the first transition phase of the avascular zone at 48 hours illustrated in figure 3a and 4a. Also, we observed the revascularization process into the avascular zone previously observed. It has been found that the avascular zone formed by heparin and angiostatic steroids became revascularized 60 hours after application. In our study, taxol-treated avascular zones did not revascularize for at least 7 days after application implying a more potent long-term effect.

Reference <u>EXAMPLE 2</u>

ENCAPSCLATION OF SURAMIN

[0060] One milliliter of 5% ELVAX (poly(ethylene-vinyl acetate) crosslinked with 5% vinyl acetate) in dichloromethane ("DCM") is mixed with a fixed weight of sub-micron ground sodium suramin. This mixture is injected into 5 ml of 5% Polyvinyl Alcohol ("PVA") in water in a 30 ml flat bottomed test tube. Tubes containing different weights of the drug are then suspended in a multi-sample water bath at 40° for 90 minutes with automated stirring. The mixes are removed, and microsphere samples taken for size analysis. Tubes are centrifuged at 1000g for 5 min. The PVA supernatant is removed and saved for analysis (nonencapsulated drug). The microspheres are then washed (vortexed) in 5 ml of water and recentrifuged. The 5 ml wash is saved for analysis (surface bound drug). Microspheres are then wetted in 50 ul of methanol, and vortexed in 1 ml of DCM to dissolve the ELVAX. The microspheres are then warmed to 40°C, and 5 ml of 50°C water is slowly added with stirring. This procedure results in the immediate evaporation of DCM, thereby causing the release of sodium suramin into the 5 ml of water. All three 5 ml samples were then assayed for drug content.

[0061] Sodium suramin absorbs uv/vis with a lambda max of 312nm. The absorption is linear in the 0 to 100 ug/ml range in both water and 5% PVA. The drug fluoresces strongly with an excitation maximum at 312nm, and emission maximum at 400nm. This fluorescence is quantifiable in the 0 to 25 ug/ml range.

[0062] Results are shown in Figures 5-10. Briefly, the size distribution of microspheres appears to be unaffected by inclusion of the drug in the DCM (see Figures 5 and 6). Good yields of microspheres in the 20 to 60 μm range may be obtained.

[0063] The encapsulation of suramin is very low (<1%) (see Figure 8). However as the weight of drug is increased in the DCM the total amount of drug encapsulated increased although the % encapsulation decreased. As is shown in Figure 7, 50ug of drug may be encapsulated in 50 mg of ELVAX. Encapsulation of sodium suramin in 5% PVA containing 10% NaCl is shown in Figures 9-10.

Reference <u>Example 3</u>

ENCAPSULATION OF TAXOL

[0064] Five hundred micrograms of either taxol or baccatin (a taxol analog, available from Inflazyme Pharmaceuticals Inc., Vancouver, British Columbia, Canada) are dissolved in 1 ml of a 50:50 ELVAX:poly-1-lactic acid mixture in dcm. Microspheres are then prepared in a dissolution machine (Six-spindle dissolution tester, VanderKanp, Van Ken Industries Inc., U.S.A.) in triplicate at 200 rpm, 42°C, for 3 hours. Microspheres so prepared are washed twice in water and sized on the microscope..

[0065] Determination of taxol encapsulation is undertaken in a uv/vis assay (uv/vis lamda max. at 237 nm, fluorescence assay at excitation 237, emission at 325 nm; Fluorescence results are presented in square brackets [ ]). Utilising the procedures described above, 58 μg (+/-12 μg) [75 μg (+ /-25 μg)] of taxol may be encapsulated from a total 500 μg of starting material. This represents 12% (+/-2.4%) [15% (+/-5%)] of the original weight, or 1.2% (+/-0.25%) [1.5% (+/-0.5%)] by weight of the polymer. After 18 hours of tumbling in an oven at 37°C, 10.3% (+/-10%) [6% (+/-5.6%)] of the total taxol had been released from the microspheres.

[0066] For baccatin, 100 +/-15 μg [83 +/-23μg] of baccatin can be encapsulated from a total of 500 μg starting material. This represents a 20% (+/-3%) [17% (+/-5%) of the original weight of baccatin, and 2% (+/-0.3%) [1.7% (+/-0.5%)] by weight of the polymer. After 18 hours of tumbling in an oven at 37°C, 55% (+ /-13%) [60% (+/- 23%)] of the baccatin is released from the microspheres.

Reference <u>EXAMPLE 4</u>

ANALYSIS OF SURGICAL PASTE CONTAINING ANTI-ANGIOGENIC COMPOSITIONS

[0067] Fisher rats weighing approximately 300 grams are anesthetized, and a 1 cm transverse upper abdominal incision is made. Two-tenths of a milliliter of saline cantaining 1×10^6 live 9L gliosarcoma cells (eluted immediately prior to use from tissue culture) are injected into 2 of the 5 hepatic lobes by piercing a 27 gauge needle 1 cm through the liver capsule. The abdominal wound is closed with 6.0 resorptible suture and skin clips and the GA terminated.

[0068] After 2 weeks, the tumor deposits will measure approximately 1 cm. At this time, both hepatic tumors are resected and the bare margin of the liver is packed with a hemostatic agent, The rats are divided into two groups: half is administered polymeric carrier alone, and the other half receives an anti-angiogenic composition.

[0069] Rats are sacrificed 2, 7, 14, 21 and 84 days post hepatic resection. In particular, the rats are euthanized by injecting Euthanyl into the dorsal vein of the tail. The liver, spleen, and both lungs are removed, and histologic analysis

is performed in order to study the tumors for evidence of anti-angiogenic activity.

Reference EXAMPLE 5

TRANSPLANTATION OF BILLARY STENTS IN RATS

[0070] General anesthetic is administered to 300 gram Fisher rats. A 1 cm transverse incision is then made in the upper abdomen, and the liver identified. In the most superficial lobe, 0.2 ml of saline containing 1 million cells of 9L gliosarcoma cells (eluted from tissue culture immediately prior to use) is injected via a 27 gauge needle to a depth of 1 cm into the liver capsule. Hemostasis is achieved after removal of the needle by placing a pledget of gelfoam at the puncture sites. Saline is injected as the needle is removed to ensure no spillage of cells into the peritoneal cavity or along the needle track. The general anesthetic is terminated, and the animal returned to the animal care center and placed on a normal diet.

[0071] Two weeks later, general anesthetic is administered, and utilizing aseptic precautions, the hepatic lobe containing the tumor is identified through a midline incision. A 16 gauge angiographic needle is then inserted through the hepatic capsule into the tumor, a 0.038-inch guidewire passed through the needle, and the needle withdrawn over the guidewire. A number 5 French dilator is passed over the guide into the tumor and withdrawn. A number 5 French delivery catheter is then passed over the wire containing a self-expanding stainless steel Wallstent (5 mm in diameter and 1 cm long). The stent is deployed into the tumor and the guidewire delivery catheter is removed. One-third of the rats have a conventional stainless steel stent inserted into the tumor, one-third a stainless steel stent coated with polymer, and one third a stent coated with the polymer-anti-angiogenic factor compound. The general anesthetic is terminated and the rat returned to the animal care facility.

[0072] A plain abdominal X-ray is performed at 2 days in order to assess the degree of stent opening. Rats are sacrificed at 2, 7, 14, 28 and 56 days post-stent insertion by injecting Euthanyl, and their livers removed en *bloc* once euthanasia is confirmed. After fixation in formaldehyde for 48 hours, the liver is sectioned at 0.5 mm internal; including severing the stent transversely using a fresh blade for each slice. Histologic sections stained with H and E are then analyzed to assess the degree of tumor ingrowth into the stent lumen.

Reference EXAMPLE 6

MANUFACTURE OF MICROSPHERES

[0073] Equipment which is preferred for the manufacture of Microspheres described below include: 200 ml water jacketed beaker (Kimax or Pyrex), Haake circulating water bath, overhead stirrer and controller with 2 inch diameter (4 blade, propeller type stainless steel stirrer Fisher brand), 500 ml glass beaker, hot plate/stirrer (Corning brand), 4 X 50 ml polypropylene centrifuge tubes (Nalgene), glass scintillation vials with plastic insert caps, table top centrifuge (GPR Beckman), high speed centrifuge- floor model (JS 21 Beckman), Mettler analytical balance (AJ 100, 0.1 mg), Mettler digital top loading balance (AE 163, 0.01 mg), automatic pipetter (Gilson). Reagents include Polycaprolactone ("PCL" - mol wt 10,000 to 20,000; Polysciences, Warrington Pennsylvania. USA), "washed" Ethylene Vinyl Acetate ("EVA" washed so as to remove the anti-oxidant BHT), Poly(DL)lactic acid ("PLA" - mol wt 15,000 to 25,000; Polysciences), Polyvinyl Alcohol ("PVA" - mol wt 124.000 to 186,000; 99% hydrolyzed; Aldrich Chemical Co., Milwaukee WI, USA), Dichloromethane ("DCM" or "methylene chloride"; HPLC grade Fisher scientific), and distilled water.

A. Preparation of 5% (w/v) Polymer Solutions

[0074] Depending on the polymer solution being prepared, 1.00 g of PCL or PLA, or 0.50 g each of PLA and washed EVA is weighed directly into a 20 ml glass scintillation vial. Twenty milliliters of DCM is then added, and the vial tightly capped. The vial is stored at room temperature (25°C) for one hour (occasional shaking may be used), or until all the polymer has dissolved (the solution should be clear). The solution may be stored at room temperature for at least two weeks.

B. Preparation of 5% (w/v) Stock Solution of PVA

[0075] Twenty-five grams of PVA is weighed directly into a 600 ml glass beaker. Five hundred milliliters of distilled water is added, along with a 3 inch Teflon coated stir bar. The beaker is covered with glass to decrease evaporation losses, and placed into a 2000 ml glass beaker containing 300 ml of water (which acts as a water bath). The PVA is stirred at 300 rpm at 85°C (Coming hot plate/stirrer) for 2 hours or until fully dissolved. Dissolution of the PVA may be determined by a visual check; the solution should be clear. The solution is then transferred to a glass screw top storage

container and stored at 4°C for a maximum of two months. The solution, however should be warmed to room temperature before use or dilution.

C. Procedure for Producing Microspheres

[0076]   Based on the size of microspheres being made (see Table 1), 100 ml of the PVA solution (concentrations given in Table III) is placed into the 200 ml water jacketed beaker. Haake circulating water bath is connected to this beaker and the contents are allowed to equilibrate at 27°C (+/-10°C) for 10 minutes. Based on the size of microspheres being made (see Table III), the start speed of the overhead stirrer is set, and the blade of the overhead stirrer placed half way down in the PVA solution. The stirrer is then started, and 10 ml of polymer solution (polymer solution used based on type of microspheres being produced) is then dripped into the stirring PVA over a period of 2 minutes using a 5 ml automatic pipetter. After 3 minutes the stir speed is adjusted (see Table III), and the solution stirred for an additional 2.5 hours. The stirring blade is then removed from the microsphere preparation, and rinsed with 10 ml of distilled water so that the rinse solution drains into the microsphere preparation. The microsphere preparation is then poured into a 500 ml beaker, and the jacketed water bath washed with 70 ml, of distilled water, which is also allowed to drain into the microsphere preparation. The 180 ml microsphere preparation is then stirred with a glass rod, and equal amounts are poured into four polypropylene 50 ml centrifuge tubes. The tubes are then capped, and centrifuged for 10 minutes (force given in Table 1). A 5 ml automatic pipetter or vacuum suction is then utilized to draw 45 ml of the PVA solution off of each microsphere pellet.

TABLE III

PVA concentrations, stir speeds, and centrifugal force requirements for each diameter range of microspheres.			
PRODUCTION STAGE	MICROSPHERE DIAMETER RANGES		
	30 μm to 100 μm	10 μm to 30 μm	0.1 μm to 3 μm
PVA Concentration	25% (w/v) (*i.e.,* dilute 5% stock with distilled water	5% (w/v) (*i.e.,* undiluted stock)	3.5% (w/v) (*i.e,* dilute 5% stock with distilled water
Starting Stir Speed	500 rpm +/- 50 rpm	500 rpm +/- 50 rpm	3000 rpm +/- 200 rpm
Adjusted Stir Speed	500 rpm + /- 50 rpm	500 rpm + /- 50 rpm	2500 rpm +/- 200 rpm
Centrifuge Force	1000 g + /- 100 g (Table top model)	1000 g + /- 100 g (Table top model)	10 000 g +/-1000 g (High speed model)

[0077]   Five milliliters of distilled water is then added to each centrifuge tube, which is then vortexed to resuspend the microspheres. The four microsphere suspensions are then pooled into one centrifuge tube along with 20 ml of distilled water, and centrifuged for another 10 minutes (force given in Table 1). This process is repeated two additional times for a total of three washes. The microspheres are then centrifuged a final time, and resuspended in 10 ml of distilled water. After the final wash, the microsphere preparation is transferred into a preweighed glass scintillation vial. The vial is capped, and left overnight at room temperature (25°C) in order to allow the microspheres to sediment out under gravity. Microspheres which fall in the size range of 0.1 um to 3 um do not sediment out under gravity, so they are left in the 10 ml suspension.

D. Drying of 10 μm to 30 μm or 30 μm to 100 μm Diameter Microspheres

[0078]   After the microspheres have sat at room temperature overnight, a 5 ml automatic pipetter or vacuum suction is used to draw the supernatant off of the sedimented microspheres. The microspheres are allowed to dry in the uncapped vial in a drawer for a period of one week or until they are fully dry (vial at constant weight). Faster drying may be accomplished by leaving the uncapped vial under a slow stream of nitrogen gas (flow approx. 10 ml/min.) in the fume hood. When fully dry (vial at constant weight), the vial is weighed and capped. The labelled, capped vial is stored at room temperature in a drawer. Microspheres are normally stored no longer than 3 months.

E. Drying of 0.1 μm to 3 μm Diameter Microspheres

[0079]   This size range of microspheres will not sediment out, so they are left in suspension at 4°C for a maximum of four weeks. To determine the concentration of microspheres in the 10 ml suspension, a 200 μl sample of the suspension

is pipetted into a 1.5 ml preweighed microfuge tube. The tube is then centrifuged at 10,000 g (Eppendorf table top microfuge), the supernatant removed, and the tube allowed to dry at 50°C overnight. The tube is then reweighed in order to determine the weight of dried microspheres within the tube.

F. Manufacture of Taxol Loaded Microsphere

[0080]   In order to prepare taxol containing microspheres, an appropriate amount of weighed taxol (based upon the percentage of taxol to be encapsulated) is placed directly into a 20 ml glass scintillation vial. Ten milliliters of an appropriate polymer solution is then added to the vial containing the taxol, which is then vortexed until the taxol has dissolved.

[0081]   Microspheres containing taxol may then be produced essentially as described above in steps (C) through (E).

EXAMPLE 7

MANUFACTURE OF STENT COATING

[0082]   Reagents and equipment which are utilized within the following experiments include (medical grade stents obtained commercially from a variety of manufacturers; *e.g.*, the "Strecker" stent) and holding apparatus, 20 ml glass scintillation vial with cap (plastic insert type), TLC atomizer, Nitrogen gas tank, glass test tubes (various sizes from 1 ml and up), glass beakers (various sizes), Pasteur pipette, tweezers, Polycaprolactone ("PCL" - mol wt 10,000 to 20,000; Polysciences), Taxol (Sigma Chemical Co., St. Louis, Mo., 95% purity), Ethylene vinyl acetate ("EVA" - washed - see previous), Poly(DL)lactic acid ("PLA" - mol wt 15,000 to 25,000; Polysciences), dichloromethane ("DCM" - HPLC grade, Fisher Scientific).

A. Procedure for Sprayed Stents

[0083]   The following describes a typical method using a 3 mm crimped diameter interleaving metal wire stent of approximately 3 cm length. For larger diameter stents, larger volumes of polymer/drug solution are used.

[0084]   Weigh sufficient polymer directly into a 20 ml glass scintillation vial and add sufficient DCM to achieve a 2% w/v solution. Cap the vial and mix the solution to dissolve the polymer (hand shaking). Assemble the stent in a vertical orientation. This can be accomplished using a piece of nylon and tying the stent to a retort stand. Position this stent holding apparatus 6 to 12 inches above the fume hood floor on a suitable support (*e.g.*, inverted 2000 ml glass beaker) to enable horizontal spraying. Using an automatic pipette, transfer a suitable volume (minimum 5 ml) of the 2% polymers solution to a separate 20 ml glass scintillation vial. Add an appropriate amount of taxol to the solution and dissolve it by hand shaking the capped vial.

[0085]   To prepare for spraying, remove the cap of this vial and dip the barrel (only) of an TLC atomizer into the polymer solution. Note that the reservoir of the atomizer need not be used in this procedure: the 20 ml glass vial acts as a reservoir. Connect the nitrogen tank to the gas inlet of the atomizer. Gradually increase the pressure until atomization and spraying begins. Note the pressure and use this pressure throughout the procedure. To spray the stent use 5 second oscillating sprays with a 15 second dry time between sprays. After 5 sprays, rotate the stent 90° and spray that portion of the stent. Repeat until all sides of the stent have been sprayed. During the dry time, finger crimp the gas line to avoid wastage of the spray. Spraying is continued until a suitable amount of polymer is deposited on the stents. The amount may be based on the specific stent application *in vivo.* To determine the amount, weigh the stent after spraying has been completed and the stent has dried. Subtract the original weight of the stent from the finished weight and this produces the amount of polymer (plus taxol) applied to the stent. Store the coated stent in a sealed container.

B. Procedure for Dipped Stents

[0086]   The following describes a typical method using a 3 mm crimped diameter interleaving metal wire stent of approximately 3 cm length. For larger diameter stents, larger volumes of polymer/drug solution are used in larger sized test tubes.

[0087]   Weigh 2 g of EVA into a 20 ml glass scintillation vial and add 20 ml of DCM. Cap the vial and leave it for 2 hours to dissolve (hand shake the vial frequently to assist the dissolving process). Weigh a known weight of taxol directly into a 1 ml glass test tube and add 0.5 ml of the polymer solution. Using a glass Pasteur pipette, dissolve the taxol by gently pumping the polymer solution. Once the taxol is dissolved, hold the test tube in a near horizontal position (the sticky polymer solution will not flow out). Using tweezers, insert the stent into the tube all the way to the bottom. Allow the polymer solution to flow almost to the mouth of the test tube by angling the mouth below horizontal and then restoring the test tube to an angle slightly above the horizontal. While slowly rotating the stent in the tube, slowly remove the stent (approximately 30 seconds).

[0088] Hold the stent in a vertical position to dry. Some of the *sealed* perforations may *pop* so that a hole exists in the continuous sheet of polymer. This may be remedied by repeating the previous dipping procedure, however repetition of the procedure can also lead to further popping and a general uneven build up of polymer. Generally, it is better to dip the stent just once and to cut out a section of stent that has *no popped* perforations. Store the dipped stent in a sealed container.

[0089] Reference EXAMPLE 8

Procedure for Producing Film

[0090] The term film refers to a polymer formed into one of many geometric shapes. The film may be a thin, elastic sheet of polymer or a 2 mm thick disc of polymer. This film is designed to be placed on exposed tissue so that any encapsulated drug is released from the polymer over a long period of time at the tissue site. Films may be made by several processes, including for example, by casting, and by spraying.

[0091] In the casting technique, polymer is either melted and poured into a shape or dissolved in dichloromethane and poured into a shape. The polymer then either solidifies as it cools or solidifies as the solvent evaporates, respectively.. In the spraying technique, the polymer is dissolved in solvent and sprayed onto glass, as the solvent evaporates the polymer solidifies on the glass. Repeated spraying enables a build up of polymer into a film that can be peeled from the glass.

[0092] Reagents and equipment which were utilized within these experiments include a small beaker, Corning hot plate stirrer, casting moulds (*e.g.,* 50 ml centrifuge tube caps) and mould holding apparatus, 20 ml glass scintillation vial with cap (Plastic insert type), TLC atomizer, Nitrogen gas tank, Polycaprol8actone ("PCL" - mol wt 10,000 to 20,000; Polysciences), Taxol (Sigma 95% purity), Ethanol, "washed" (see previous) Ethylene vinyl acetate ("EVA"), Poly(DL) lactic acid ("PLA" - mol wt 15,000 to 25,000; Polysciences), Dichloromethane (HPLC grade Fisher Scientific).

1. *Procedure for Producing Films- Melt Casting*

[0093] Weigh a known weight of PCL directly into a small glass beaker. Place the beaker in a larger beaker containing water (to act as a water bath) and put it on the hot plate at 70°C for 15 minutes or until the polymer has fully melted. Add a known weight of drug to the melted polymer and stir the mixture thoroughly. To aid dispersion of the drug in the melted PCL, the drug may be suspended/dissolved in a small volume (< 10% of the volume of the melted PCL) of 100% ethanol. This ethanol suspension is then mixed into the melted polymer. Pour the melted polymer into a mould and let it to cool. After cooling, store the film in a container.

2. *Procedure for Producing Films - Solvent Casting*

[0094] Weigh a known weight of PCL directly into a 20 ml glass scintillation vial and add sufficient DCM to achieve a 10% w/v solution. Cap the vial and mix the solution. Add sufficient taxol to the solution to achieve the desired final taxol concentration. Use hand shaking or vortexing to dissolve the taxol in the solution. Let the solution sit for one hour (to diminish the presence of air bubbles) and then pour it slowly into a mould. The mould used is based on the shape required. Place the mould in the fume hood overnight. This will allow the DCM to evaporate. Either leave the film in the mould to store it or peel it out and store it in a sealed container.

3. *Procedure for Producing Films - Sprayed*

[0095] Weigh sufficient polymer directly into a 20 ml glass scintillation vial and add sufficient DCM to achieve a 2% w/v solution. Cap the vial and mix the solution to dissolve the polymer (hand shaking). Assemble the moulds in a vertical orientation in a suitable mould holding apparatus in the fume hood. Position this mould holding apparatus 6 to 12 inches above the fume hood floor on a suitable support (*e.g.*, inverted 2000 ml glass beaker) to enable horizontal spraying. Using an automatic pipette, transfer a suitable volume (minimum 5 ml) of the 2% polymer solution to a separate 20 ml glass scintillation vial. Add sufficient taxol to the solution and dissolve it by hand shaking the capped vial. To prepare for spraying, remove the cap of this vial and dip the barrel (only) of an TLC atomizer into the polymer solution. Note: the reservoir of the atomizer is not used in this procedure - the 20 ml glass viral acts as a reservoir.

[0096] Connect the nitrogen tank to the gas inlet of the atomizer. Gradually increase the pressure until atomization and spraying begins. Note the pressure and use this pressure throughout the procedure. To spray the moulds use 5 second oscillating sprays with a 15 second dry time between sprays. During the dry time, finger crimp the gas line to avoid wastage of the spray. Spraying is continued until a suitable thickness of polymer is deposited on the mould. The thickness is based on the request. Leave the sprayed films attached to the moulds and store in sealed containers.

Reference <u>EXAMPLE 9</u>

CONTROLLED DELIVERY OF TAXOL FROM MICROSPHERES COMPOSED OF A BLEND OF ETHYLENE-VINYL-ACETATE COPOLYMER AND POLY (D,L.LACTIC ACID). IN VIVO TESTING OF THE MICROSPHERES ON THE CAM ASSAY

[0097] This example describes the preparation of taxol-loaded microspheres composed of a blend of biodegradable poly (d,l-lactic acid) (PLA) polymer and nondegradable ethylene-vinyl acetate (EVA) copolymer. In addition, the *in vitro* release rate and anti-angiogenic activity of taxol released from microspheres placed on a CAM are demonstrated.

[0098] Reagents which were utilized in these experiments include taxol, which is purchased from Sigma Chemical Co. (St. Louis, MO); PLA (molecular weight 15,000-25,000) and EVA (60% vinyl acetate) (purchased from Polysciences (Warrington, PA); polyvinyl alcohol (PVA) (molecular weight 124,000-186,000, 99% hydrolysed, purchased from Aldrich Chemical Co. (Milwaukee, WI)) and Dichloromethane (DCM) (HPLC grade, obtained from Fisher Scientific Co). Distilled water is used throughout.

A. <u>Preparation of microspheres</u>

[0099] Microspheres are prepared essentially as described in Example 8 utilizing the solvent evaporation method.. Briefly, 5% w/v polymer solutions in 20 mL DCM are prepared using blends of EVA:PLA between 35:65 to 90:10. To 5 mL of 2.5% w/v PVA in water in a 20 mL glass vial is added 1 mL of the polymer solution dropwise with stirring. Six similar vials are assembled in a six position overhead stirrer, dissolution testing apparatus (Vanderkamp) and stirred at 200 rpm. The temperature of the vials is increased from room temperature to 40°C over 15 min and held at 40°C for 2 hours. Vials are centrifuged at 500xg and the microspheres washed three times in water. At some EVA:PLA polymer blends, the microsphere samples aggregated during the washing stage due to the removal of the dispersing or emulsifying agent, PVA. This aggregation effect could be analyzed semi-quantitatively since aggregated microspheres fused and the fused polymer mass floated on the surface of the wash water. This surface polymer layer is discarded during the wash treatments and the remaining, pelleted microspheres are weighed.
The % aggregation is determined from

% aggregation = $1 - \dfrac{\text{(weight of pelleted microspheres)}}{\text{initial polymer weight}} \times 100$

[0100] Taxol loaded microspheres (0.6% w/w taxol) are prepared by dissolving the taxol in the 5% w/v polymer solution in DCM. The polymer blend used is 50:50 EVA:PLA. A "large" size fraction and "small" size fraction of microspheres are produced by adding the taxol/polymer solution dropwise into 2.5% w/v PVA and 5% w/v PVA, respectively. The dispersions are stirred at 40°C at 200 rpm for 2 hours, centrifuged and washed 3 times in water as described previously. Microspheres are air dried and samples are sized using an optical microscope with a stage micrometer. Over 300 microspheres are counted per sample. Control microspheres (taxol absent) are prepared and sized as described previously.

B. <u>Encapsulation efficiency</u>

[0101] Known weights of taxol-loaded microspheres are dissolved in 1 mL DCM, 20 mL of 40% acetonitrile in water at 50°C are added and vortexed until the DCM had been evaporated. The concentration of taxol in the 40% acetonitrile is determined by HPLC using a mobile phase of water:methanol:acetonitrile (37:5:58) at a flow rate of 1 mL/min (Beckman isocratic pump), a C8 reverse phase column (Beckman) and UV detection at 232 nm. To determine the recovery efficiency of this extraction procedure, known weights of taxol from 100-1000 μg are dissolved in 1 mL of DCM and subjected to the same extraction procedure in triplicate as described previously. Recoveries are always greater than 85% and the values of encapsulation efficiency are corrected appropriately.

C. <u>Drug release studies</u>

[0102] In 15 mL glass, screw capped tubes are placed 10 mL of 10 mM phosphate buffered saline (PBS), pH 7.4 and 35 mg taxol-loaded microspheres. The tubes are tumbled at 37°C and at given time intervals, centrifuged at 1500xg for 5 min and the supernatant saved for analysis. Microsphere pellets are resuspended in fresh PBS (10mL) at 37°C and reincubated. Taxol concentrations are determined by extraction into 1 mL DCM followed by evaporation to dryness under a stream of nitrogen, reconstitution in 1 mL of 40% acetonitrile in water and analysis using HPLC as previously described.

D. <u>Scanning Electron Microscopy (SEM)</u>

[0103] Microspheres are placed on sample holders, sputter coated with gold and micrographs obtained using a Philips

501B SEM operating at 15 kV.

E. CAM Studies

[0104] Fertilized, domestic chick embryos are incubated for 4 days prior to shell-less culturing. The egg contents are incubated at 90% relative humidity and 3% CO_2 for 2 days. On day 6 of incubation, 1 mg aliquots of 0.6% taxol loaded or control (taxol free) microspheres are placed directly on the CAM surface. After a 2 day exposure the vasculature is examined using a stereomicroscope interfaced with a video camera; the video signals are then displayed on a computer and video printed.

F. Results

[0105] Microspheres prepared from 100% EVA are freely suspended in solutions of PVA but aggregated and coalesced or fused extensively on subsequent washing in water to remove the PVA. Blending EVA with an increasing proportion of PLA produced microspheres showing a decreased tendency to aggregate and coalesce when washed in water, as described in Figure 15A. A 50:50 blend of EVA:PLA formed microspheres with good physical stability, that is the microspheres remained discrete and well suspended with negligible aggregation and coalescence.

[0106] The size range for the "small" size fraction microspheres is determined to be > 95% of the microsphere sample (by weight) between 10-30 mm and for the "large" size fraction, >95% of the sample (by weight) between 30-100 mm. Representative scanning electron micrographs of taxol loaded 50:50 EVA:PLA microspheres in the "small" and "large" size ranges are shown in Figures 15B and 15C, respectively. The microspheres are spherical with a smooth surface and with no evidence of solid drug on the surface of the microspheres. The efficiency of loading 50:50 EVA:PLA microspheres with taxol is between 95-100% at initial taxol concentrations of between 100-1000 mg taxol per 50 mg polymer. There is no significant difference (Student t-test, $p < 0.05$) between the encapsulation efficiencies for either "small" or "large" microspheres.

[0107] The time course of taxol release from 0.6% w/v loaded 50:50 EVA:PLA microspheres is shown in Figure 15D for "small" size (open circles) and "large" size (closed circles) microspheres. The release rate studies are carried out in triplicate tubes in 3 separate experiments. The release profiles are biphasic with an initial rapid release of taxol or "burst" phase occurring over the first 4 days from both size range microspheres. This is followed by a phase of much slower release. There is no significant difference between the release rates from "small" - "large" microspheres. Between 10-13% of the total taxol content of the microspheres is released in 50 days.

[0108] The taxol loaded microspheres (0.6% w/v loading) are tested using the CAM assay and the results are shown in Figure 15E. The taxol microspheres released sufficient drug to produce a zone of avascularity in the surrounding tissue (Figure 15F). Note that immediately adjacent to the microspheres ("MS" in Figures 15E and 15F) is an area in which blood vessels are completely absent (Zone I); further from the microspheres is an area of disrupted, non-functioning capillaries (Zone 2); it is only at a distance of approximately 6 mm from the microspheres that the capillaries return to normal. In CAMs treated with control microspheres (taxol absent) there is a normal capillary network architecture.

Discussion

[0109] Arterial chemoembolization is a invasive surgical technique. Therefore, ideally, a chemoembolic formulation of an anti-angiogenic and anticancer drug such as taxol would release the drug at the tumor site at concentrations sufficient for activity for a prolonged period of time, of the order of several months. EVA is a tissue compatible nondegradable polymer which has been used extensively for the controlled delivery of macromolecules over long time periods (> 100 days).

[0110] EVA is initially selected as a polymeric biomaterial for preparing microspheres with taxol dispersed in the polymer matrix. However, microspheres prepared with 100% EVA aggregated and coalesced almost completely during the washing procedure.

[0111] Polymers and copolymers based on lactic acid and glycolic acid are physiologically inert and biocompatible and degrade by hydrolysis to toxicologically acceptable products. Copolymers of lactic acid and glycolic acids have faster degradation rates than PLA and drug loaded microspheres prepared using these copolymers are unsuitable for prolonged, controlled release over several months. Dollinger and Sawan blended PLA with EVA and showed that the degradation lifetime of PLA is increased as the proportion of EVA in the blend is increased. They suggested that blends of EVA and PLA should provide a polymer matrix with better mechanical stability and control of drug release rates than PLA.

[0112] Figure 15A shows that increasing the proportion of PLA in a EVA:PLA blend decreased the extent of aggregation of the microsphere suspensions. Blends of 50% or less EVA in the EVA:PLA matrix produced physically stable microsphere suspensions in water or PBS. A blend of 50:50 EVA:PLA is selected for all subsequent studies.

[0113] Different size range fractions of microspheres could be prepared by changing the concentration of the emulsifier, PVA, in the aqueous phase. "Small" microspheres are produced at the higher PVA concentration of 5% w/v whereas "large" microspheres are produced at 25% w/v PVA. All other production variables are the same for both microsphere size fractions. The higher concentration of emulsifier gave a more viscous aqueous dispersion medium and produced smaller droplets of polymer/taxol/DCM emulsified in the aqueous phase and thus smaller microspheres. The taxol loaded microspheres contained between 95-100% of the initial taxol added to the organic phase encapsulated within the solid microspheres. The low water solubility of taxol favoured partitioning into the organic phase containing the polymer.

[0114] Release rates of taxol from the 50:50 EVA:PLA microspheres are very slow with less than 15% of the loaded taxol being released in 50 days. The initial burst phase of drug release may be due to diffusion of drug from the superficial region of the microspheres (close to the microsphere surface).

[0115] The mechanism of drug release from nondegradable polymeric matrices such as EVA is thought to involve the diffusion of water through the dispersed drug phase within the polymer, dissolution of the drug and diffusion of solute through a series of interconnecting, fluid filled pores. Blends of EVA and PLA have been shown to be immiscible or bicontinuous over a range of 30 to 70% EVA in PLA. In degradation studies in PBS buffer at 37°C, following an induction or lag period, PLA hydrolytically degraded and eroded from the EVA:PLA polymer blend matrix leaving an inactive sponge-like skeleton. Although the induction period and rate of PLA degradation and erosion from the blended matrices depended on the proportion of PLA in the matrix and on process history, there is consistently little or no loss of PLA until after 40-50 days.

[0116] Although some erosion of PLA from the 50:50 EVA:PLA microspheres may have occurred within the 50 days of the *in vitro* release rate study (Figure 15C), it is likely that the primary mechanism of drug release from the polymer blend is diffusion of solute through a pore network in the polymer matrix.

[0117] At the conclusion of the release rate study, the microspheres are analyzed from the amount of drug remaining. The values for the percent of taxol remaining in the 50 day incubation microsphere samples are 94% +/- 9% and 89% +/- 12% for "large" and "small" size fraction microspheres, respectively.

[0118] Microspheres loaded with 6mg per mg of polymer (0.6%) provided extensive inhibition of angiogenesis when placed on the CAM of the embryonic chick (Figures 15E and 15F).

Reference EXAMPLE 10

TAXOL ENCAPSULATION IN POLY(E-CAPROLACTONE) MICROSPHERES INHIBITION OF ANGIOGENESIS ON THE CAM ASSAY BY TAXOL-LOADED MICROSPHERES

[0119] This example evaluates the *in vitro* release rate profile of taxol from biodegradable microspheres of poly(e-caprolactone) and demonstrates the anti-angiogenic activity of taxol released from these microspheres when placed on the CAM.

[0120] Reagents which were utilized in these experiments include: poly(e-caprolactone) ("PCL") (molecular weight 35,000 - 45,000; purchased from Polysciences (Warrington, PA)); dichloromethane ("DCM") from Fisher Scientific Co., Canada; polyvinyl alcohol (PVP) (molecular weight 12,00 - 18,000, 99% hydrolysed) from Aldrich Chemical Co. (Milwaukee, Wis.), and taxol from Sigma Chemical Co. (St. Louis, MO). Unless otherwise stated all chemicals and reagents are used as supplied. Distilled water is used throughout.

A. Preparation of microspheres

[0121] Microspheres are prepared essentially as described in Example 8 utilizing the solvent evaporation method. Briefly, 5%w/w taxol loaded microspheres are prepared by dissolving 10 mg of taxol and 190 mg of PCL in 2 ml of DCM, adding to 100 ml of 1% PVP aqueous solution and stirring at 1000 r.p.m. at 25°C for 2 hours. The suspension of microspheres is centrifuged at 1000 x g for 10 minutes (Beckman GPR), the supernatant removed and the microspheres washed three times with water. The washed microspheres are air-dried overnight and stored at room temperature. Control microspheres (taxol absent) are prepared as described above. Microspheres containing 1% and 2% taxol are also prepared. Microspheres are sized using an optical microscope with a stage micrometer.

B. En_capsulation efficiency

[0122] A known weight of drug-loaded microspheres (about 5 mg) is dissolved in 8 ml of acetonitrile and 2 ml distilled water is added to precipitate the polymer. The mixture is centrifuged at 1000 g for 10 minutes and the amount of taxol encapsulated is calculated from the absorbance of the supernatant measured in a UV spectrophotometer (Hewlett-Packard 8452A Diode Array Spectrophotometer) at 232 nm.

C. Drug release studies

[0123] About 10 mg of taxol-loaded microspheres are suspended in 20 ml of 10 mM phosphate buffered saline, pH 7.4 (PBS) in screw-capped tubes. The tubes are tumbled end-over-end at 37°C and at given time intervals 19.5 ml of supernatant is removed (after allowing the microspheres to settle at the bottom), filtered through a 0.45 mm membrane filter and retained for taxol analysis. An equal volume of PBS is replaced in each tube to maintain sink conditions throughout the study. The filtrates are extracted with 3 x 1 ml DCM, the DCM extracts evaporated to dryness under a stream of nitrogen, redissolved in 1 ml acetonitrile and analyzed by HPLC using a mobile phase of water:methanol: acetonitrile (37:5:58) at a flow rate of 1ml min^{-1} (Beckman Isocratic Pump), a C8 reverse phase column (Beckman), and UV detection (Shimadzu SPD A) at 232 nm.

D. CAM studies

[0124] Fertilized, domestic chick embryos are incubated for 4 days prior to shell-less culturing. On day 6 of incubation, 1 mg aliquots of 5% taxol-loaded or control (taxol-free) microspheres are placed directly on the CAM surface. After a 2-day exposure the vasculature is examined using a stereomicroscope interfaced with a video camera; the video signals are then displayed on a computer and video printed.

E. Scanning electron microscopy

[0125] Microspheres are placed on sample holders, sputter-coated with gold and then placed in a Philips 501B Scanning Electron Microscope operating at 15 kV.

F. Results

[0126] The size range for the microsphere samples is between 30 - 100 mm, although there is evidence in all taxol-loaded or control microsphere batches of some microspheres falling outside this range. The efficiency of loading PCL microspheres with taxol is always greater than 95% for all drug loadings studied. Scanning electron microscopy demonstrated that the microspheres are all spherical and many showed a rough or pitted surface morphology. There appeared to be no evidence of solid drug on the surface of the microspheres.

[0127] The time courses of taxol release from 1%, 2% and 5% loaded PCL microspheres are shown in Figure 16A. The release rate profiles are biphasic. There is an initial rapid release of taxol or "burst phase" at all drug loadings. The burst phase occurred over 1-2 days at 1% and 2% taxol loading and over 3-4 days for 5% loaded microspheres. The initial phase of rapid release is followed by a phase of significantly slower drug release. For microspheres containing 1% or 2% taxol there is no further drug release after 21 days. At 5% taxol loading, the microspheres had released about 20% of the total drug content after 21 days.

[0128] Figure 16B shows CAMs treated with control PCL microspheres, and Figure 16C shows treatment with 5% taxol loaded microspheres. The CAM with the control microspheres shows a normal capillary network architecture. The CAM treated with taxol-PCL microspheres shows marked vascular regression and zones which are devoid of a capillary network.

G. Discussion

[0129] The solvent evaporation method of manufacturing taxol-loaded microspheres produced very high taxol encapsulation efficiencies of between 95-100%. This is due to the poor water solubility of taxol and its hydrophobic nature favouring partitioning in the organic solvent phase containing the polymer.

[0130] The biphasic release profile for taxol is typical of the release pattern for many drugs from biodegradable polymer matrices. Poly(e-caprolactone) is an aliphatic polyester which can be degraded by hydrolysis under physiological conditions and it is non-toxic and tissue compatible. The degradation of PCL is significantly slower than that of the extensively investigated polymers and copolymers of lactic and glycolic acids and is therefore suitable for the design of long-term drug delivery systems. The initial rapid or burst phase of taxol release is thought to be due to diffusional release of the drug from the superficial region of the microspheres (close to the microsphere surface). Release of taxol in the second (slower) phase of the release profiles is not likely due to degradation or erosion of PCL because studies have shown that under *in vitro* conditions in water there is no significant weight loss or surface erosion of PCL over a 7.5-week period. The slower phase of taxol release is probably due to dissolution of the drug within fluid-filled pores in the polymer matrix and diffusion through the pores. The greater release rate at higher taxol loading is probably a result of a more extensive pore network within the polymer matrix.

[0131] Taxol microspheres with 5% loading have been shown to release sufficient drug to produce extensive inhibition

of angiogenesis when placed on the CAM. The inhibition of blood vessel growth resulted in an avascular zone as shown in Figure 16C.

Reference EXAMPLE 11

TAXOL-LOADED POLYMERIC FILMS COMPOSED OF ETHYLENE VINYL ACETATE AND A SURFACTANT

[0132] Two types of films are prepared esentially as described in Reference Example 8. pure EVA films loaded with taxol and EVA/surfactant blend films (*i.e.*, Pluronic F127, Span 80 and Pluronic L101) loaded with taxol.

[0133] The surfactants being examined are two hydrophobic surfactants (Span 80 and Pluronic L101) and one hydrophilic surfactant (Pluronic F127). The pluroinc surfactants are themselves polymers, which is an attractive property since they can be blended with EVA to optimize various drug delivery properties. Span 80 is a smaller molecule which is in some manner dispersed in the polymer matrix, and does not form a blend.

[0134] Surfactants will be useful in modulating the release rates of taxol from films and optimizing certain physical parameters of the films. One aspect of the surfactant blend films which indicates that drug release rates can be controlled is the ability to vary the rate and extent to which the compound will swell in water. Diffusion of water into a polymer-drug matrix is critical to the release of drug from the carrier. Figures 17C and 17D show the degree of swelling of the films as the level of surfactant in the blend is altered. Pure EVA films do not swell to any significant extent in over 2 months. However, by increasing the level of surfactant added to the EVA it is possible to increase the degree of swelling of the compound, and by increasing hydrophilicity swelling can also be increased.

[0135] Results of experiments with these films are shown below in Figures 17A-E. Briefly, Figure 17A shows taxol release (in mg) over time from pure EVA films, Figure 17B shows the percentage of drug remaining for the same films. As can be seen from these two figures, as taxol loading increases (*i.e.*, percentage of taxol by weight is increase), drug release rates increase, showing the expected concentration dependence. As taxol loading is increased, the percent taxol remaining in the film also increases, indicating that higher loading may be more attractive for long-term release formulations.

[0136] Physical strength and elasticity of the films is assessed in Figure 17E. Briefly, Figure 17E shows stress/strain curves for pure EVA and EVASurfactant blend films. This crude measurement of stress demonstrates that the elasticity of films is increased with the addition of Pluronic F127, and that the tensile strength (stress on breaking) is increased in a concentration dependant manner with the addition of Pluronic F127. Elasticity and strength are important considerations in designing a film which can be manipulated for particular clinical applications without causing permanent deformation of the compound.

[0137] The above data demonstrates the ability of certain surfactant additives to control drug release rates and to alter the physical characteristics of the vehicle.

Reference EXAMPLE 12

INCORPORATING METHOXYPOLYETHYLENE GLYCOL 350 (MEPEG) INTO POLY(E-CAPROLACTONE) TO DEVELOP A FORMULATION FOR THE CONTROLLED DELIVERY OF TAXOL FROM A PASTE

[0138] Reagents and equipment which were utilized within these experiments include methoxypolyethylene glycol 350 ("MePEG" - Union Carbide, Danbury, CT). MePEG is liquid at room temperature, and has a freezing point of 10° to -5°C.

A. Preparation of a MePEG/PCL taxol-containing paste

[0139] MePEG/PCL paste is prepared by first dissolving a quantity of taxol into MePEG, and then incorporating this into melted PCL. One advantage with this method is that no DCM is required.

B. Analysis of melting point

[0140] The melting point of PCL/MePEG polymer blends may be determined by differential scanning calorimetry from 30°C to 70°C at a heating rate of 2.5°C per minute. Results of this experiment are shown in Figures 18A and 18B. Briefly, as shown in Figure 18A the melting point of the polymer blend (as determined by thermal analysis) is decreased by MePEG in a concentration dependent manner. The melting point of the polymer blends as a function of MePEG concentration is shown in Figure 18A. This lower melting point also translates into an increased time for the polymer blends to solidify from melt as shown in Figure 18B. A 30:70 blend of MePEG:PCL takes more than twice as long to solidify from the fluid melt than does PCL alone.

C. Measurement of brittleness

[0141] Incorporation of MePEG into PCL appears to produce a less brittle solid, as compared to PCL alone. As a "rough" way of quantitating this, a weighted needle is dropped from an equal height into polymer blends containing from 0% to 30% MePEG in PCL, and the distance that the needle penetrates into the solid is then measured. The resulting graph is shown as Figure 18C. Points are given as the average of four measurements +/-1 S.D.

[0142] For purposes of comparison, a sample of paraffin wax is also tested and the needle penetrated into this a distance of 7.25 mm +/- 0.3 mm.

D. Measurement of taxol release

[0143] Pellets of polymer (PCL containing 0%, 5%, 10% or 20% MePEG) are incubated in phosphate buffered saline (PBS, pH 7.4) at 37°C, and % change in polymer weight is measured over time. As can be seen in Figure 18D, the amount of weight lost increases with the concentration of MePEG originally present in the blend. It is likely that this weight loss is due to the release of MePEG from the polymer matrix into the incubating fluid. This would indicate that taxol will readily be released from a MePEG/PCL blend since taxol is first dissolved in MePEG before incorporation into PCL.

E. Effect of varying quantities of MePEG on taxol release

[0144] Thermopastes are made up containing between 0.8% and 20% MePEG in PCL. These are loaded with 1% taxol. The release of taxol over time from 10 mg pellets in PBS buffer at 37°C is monitored using HPLC. As is shown in Figure 18E, the amount of MePEG in the formulation does not affect the amount of taxol that is released.

F. Effect of varying quantities of taxol on the total amount of taxol released from a 20% MePEG/PCL blend

[0145] Thermopastes are made up containing 20% MePEG in PCL and loaded with between 0..2% and 10% taxol. The release of taxol over time is measured as described above. As shown in Figure 18F, the amount of taxol released over time increases with increased taxol loading. When plotted as the percent total taxol released, however, the order is reversed (Figure 18G). This gives information about the residual taxol remaining in the paste and, if assumptions are made about the validity of extrapolating this data, allows for a projection of the period of time over which taxol will be released from the 20% MePEG Thermopaste.

G. Strength analysis of various MePEG/PCL blends

[0146] A CT-40 mechanical strength tester is used to measure the strength of solid polymer "tablets" of diameter 0.88 cm and an average thickness of 0.560 cm. The polymer tablets are blends of MePEG at concentrations of 0%, 5%, 10% or 20% in PCL.

[0147] Results of this test are shown in Figure 18H, where both the tensile strength and the time to failure are plotted as a function of %MePEG in the blend. Single variable ANOVA indicated that the tablet thicknesses within each group are not different. As can be seen from Figure 18H, the addition of MePEG into PCL decreased the hardness of the resulting solid..

Reference EXAMPLE 13

EFFECT OF TAXOL-LOADED THERMOPASTE ON ANGIOGENESIS *IN VIVO*

[0148] Fertilized, domestic chick embryos were incubated for 4 days prior to shell-less culturing as described in Example 2. The egg contents are removed from the shell and emptied into round-bottom sterilized glass bowls and covered with petri dish covers.

[0149] Taxol is incorporated into thermopaste at concentrations of 5%, 10%, and 20% (w/v) essentially as described above (see Example 10), and used in the following experiments. Dried cut thermopaste is then heated to 60°C and pressed between two sheets of parafilm, flattening it, and allowing it to cool. Six embryos received 20% taxol-loaded thermopaste and 6 embryos received unloaded thermopaste prepared in this manner. One embryo died in each group leaving 5 embryos in each of the control and treated groups.

[0150] Unloaded thermopaste and thermopaste containing 20% taxol was also heated to 60°C and placed directly on the growing edge of each CAM at day 6 of incubation; two embryos each were treated in this manner.

[0151] There was no observable difference in the results obtained using the different methods of administration,

indicating that the temperature of the paste at the time of application was not a factor in the outcome.

[0152] Thermopaste with 10% taxol was applied to 11 CAMs and unloaded thermopaste was applied to an additional 11 CAMs, while 5% taxol-loaded thermopaste was applied to 10 CAMs and unloaded thermopaste was applied to 10 other control CAMs. After a 2 day exposure (day 8 of incubation) the vasculature was examined with the aid of a stereomicroscope. Liposyn II, a white opaque solution, was injected into the CAM to increase the visibility of the vascular details.

[0153] In the embryos treated with 5% taxol-loaded paste, only 2 animals demonstrated maximum inhibition of angiogenesis, while the remaining 8 were only marginally affected. Of the animals treated with 10% taxol-loaded thermopaste only 2 showed maximal inhibition while the other 9 were only marginally affected.

[0154] The 20% taxol-loaded thermopaste showed extensive areas of avascularity (see Figure 19B) in all 5 of the CAMs receiving this treatment. The highest degree of inhibition was defined as a region of avascularity covering 6 mm by 6 mm in size. All of the CAMs treated with 20% taxol-loaded thermopaste displayed this degree of angiogenesis inhibition.

[0155] By comparison, the control (unloaded) thermopaste did not inhibit angiogenesis on the CAM (see Figure 19A); this higher magnification view (note that the edge of the paste is seen at the top of the image) demonstrates that the vessels adjacent to the paste are unaffected by the thermopaste. This suggests that the effect observed is due to the sustained release of taxol and is not due to the polymer itself or due to a secondary pressure effect of the paste on the developing vasculature.

[0156] This study demonstrates that thermopaste releases sufficient quantities of angiogenesis inhibitor (in this case taxol) to inhibit the normal development of the CAM vasculature.

Reference EXAMPLE 14

EFFECT OF TAXOL-LOADED THERMOPASTE ON TUMOR GROWTH AND TUMOUR ANGIOGENESIS *IN VIVO*

[0157] Fertilized domestic chick embryos are incubated for 3 days prior to having their shells removed. The egg contents are emptied by removing the shell located around the airspace, severing the interior shell membrane, perforating the opposite end of the shell and allowing the egg contents to gently slide out from the blunted end. The contents are emptied into round-bottom sterilized glass bowls, covered with petri dish covers and incubated at 90% relative humidity and 3% carbon dioxide (see Reference Example 1).

[0158] MDAY-D2 cells (a murine lymphoid tumor) is injected into mice and allowed to grow into tumors weighing 0.5-1.0 g. The mice are sacrificed, the tumor sites wiped with alcohol, excised, placed in sterile tissue culture media, and diced into 1 mm pieces under a laminar flow hood. Prior to placing the dissected tumors onto the 9-day old chick embryos, CAM surfaces are gently scraped with a 30 gauge needle to insure tumor implantation. The tumors are then placed on the CAMs after 8 days of incubation (4 days after deshelling), and allowed to grow on the CAM for four days to establish a vascular supply, Four embryos are prepared utilizing this method, each embryo receiving 3 tumors. For these embryos, one tumor receives 20% taxol-loaded thermopaste, the second tumor unloaded thermopaste, and the third tumor no treatment. The treatments are continued for two days before the results were recorded.

[0159] The explanted MDAY-D2 tumors secrete angiogenic factors which induce the ingrowth of capillaries (derived from the CAM) into the tumor mass and allow it to continue to grow in size. Since all the vessels of the tumor are derived from the CAM, while all the tumor cells are derived from the explant, it is possible to assess the effect of therapeutic interventions on these two processes independently. This assay has been used to determine the effectiveness of taxol-loaded thermopaste on: (a) inhibiting the vascularization of the tumor and (b) inhibiting the growth of the tumor cells themselves.

[0160] Direct *in vivo* stereomicroscopic evaluation and histological examination of fixed tissues from this study demonstrated the following. In the tumors treated with 20% taxol-loaded thermopaste, there was a reduction in the number of the blood vessels which supplied the tumor (see Figures 20C and 20D), a reduction in the number of blood vessels within the tumor, and a reduction in the number of blood vessels in the periphery of the tumor (the area which is typically the most highly vascularized in a solid tumor) when compared to control tumors. The tumors began to decrease in size and mass during the two days the study was conducted. Additionally, numerous endothelial cells were seen to be arrested in cell division indicating that endothelial cell proliferation had been affected. Tumor cells were also frequently seen arrested in mitosis. All 4 embryos showed a consistent pattern with the 20% taxol-loaded thermopaste suppressing tumor vascularity while the unloaded thermopaste had no effect.

[0161] By comparison, in CAMs treated with unloaded thermopaste, the tumors were well vascularized with an increase in the number and density of vessels when compared to that of the normal surrounding tissue, and dramatically more vessels than were observed in the tumors treated with taxol-loaded paste. The newly formed vessels entered the tumor from all angles appearing like spokes attached to the center of a wheel (see Figures 20A and 20B). The control tumors continued to increase in size and mass during the course of the study. Histologically, numerous dilated thin-walled

capillaries were seen in the periphery of the tumor and few endothelial were seen to be in cell division. The tumor tissue was well vascularized and viable throughout.

[0162] As an example, in two similarly-sized (initially, at the time of explantation) tumors placed on the same CAM the following data was obtained. For the tumor treated with 20% taxol-loaded thermopaste the tumor measured 330 mm x 597 mm; the immediate periphery of the tumor has 14 blood vessels, while the tumor mass has only 3-4 small capillaries. For the tumor treated with unloaded thermopaste the tumor size was 623 mm x 678 mm; the immediate periphery of the tumor has 54 blond vessels, while the tumor mass has 12-14 small blood vessels. In addition, the surrounding CAM itself contained many more blood vessels as compared to the area surrounding the taxol-treated tumor.

[0163] This study demonstrates that thermopaste releases sufficient quantities of angiogenesis inhibitor (in this case taxol) to inhibit the pathological angiogenesis which accompanies tumor growth and development. Under these conditions angiogenesis is maximally stimulated by the tumor cells which produce angiogenic factors capable of inducing the ingrowth of capillaries from the surrounding tissue into the tumor mass.. The 20% taxol-loaded thermopaste is capable of blocking this process and limiting the ability of the tumor tissue to maintain an adequate blood supply. This results in a decrease in the tumor mass both through a cytotoxic effect of the drug on the tumor cells themselves and by depriving the tissue of the nutrients required for growth and expansion.

Reference EXAMPLE 15

EFFECT OF ANGIOGENESIS INHIBITOR-LOADED THERMOPASTE ON TUMOR GROWTH *In Vivo* IN A MURINE TUMOR MODEL

[0164] The murine MDAY-D2 tumor model may be used to examine the effect of local slow release of the chemotherapeutic and anti-angiogenic compounds such as taxol on tumor growth, tumor metastasis, and animal survival. The MDAY-D2 tumor cell line is grown in a cell suspension consisting of 5% Fetal Calf Serum in alpha mem media. The cells are incubated at 37°C in a humidified atmosphere supplemented with 5% carbon dioxide, and are diluted by a factor of 15 every 3 days until a sufficient number of cells are obtained. Following the incubation period the cells are examined by light microscopy for viability and then are centrifuged at 1500 rpm for 5 minutes. PBS is added to the cells to achieve a dilation of 1,000,000 cells per ml.

[0165] Ten week old DBA/2j female mice are acclimatized for 3-4 days after arrival. Each mouse is then injected subcutaneously in the posteriolateral flank with 100,000 MDAY-D2 cells in 100 ml of PBS. Previous studies have shown that this procedure produces a visible tumor at the injection site in 3-4 days, reach a size of 1.0-1.7g by 14 days, and produces visible metastases in the liver 19-25 days post-injection. Depending upon the objective of the study a therapeutic intervention can be instituted at any point in the progression of the disease.

[0166] Using the above animal model, 20 mice are injected with 140,000 -MDAY-D2 cells s.c. and the tumors allowed to grow. On day 5 the mice are divided into groups of 5. The tumor site was surgically opened under anesthesia, the local region treated with the drug-loaded thermopaste or control thermopaste without disturbing the existing tumor tissue, and the wound was closed. The groups of 5 received either no treatment (wound merely closed), polymer (PCL) alone, 10% taxol-loaded thermopaste, or 20% taxol-loaded thermopaste (only 4 animals injected) implanted adjacent to the tumor site. On day 16, the mice were sacrificed, the tumors were dissected and examined (grossly and histologically) for tumor growth, tumor metastasis, local and systemic toxicity resulting from the treatment, effect on wound healing, effect a tumor vascularity, and condition of the paste remaining at the incision site.

[0167] The weights of the tumors for each animal is shown in the table below:

Table IV

Tumor Weights (gm)

Animal No.	Control (empty)	Control (PCL)	10% Taxol Thermopaste	20% Taxol Thermopaste
1	1.387	1.137	0.487	0.114
2	0.589	0.763	0.589	0.192
3	0.461	0.525	0.447	0.071
4	0.606	0.282	0.274	0.042
5	0.353	0.277	0.362	
Mean	0.6808	0.6040	0.4318	0.1048
Std. Deviation	0.4078	0.3761	0.1202	0.0653
P Value		0.7647	0.358	0.036

Thermopaste loaded with 20% taxol reduced tumor growth by over 85% (average weight 0.105) as compared to control animals (average weight 0.681). Animals treated with thermopaste alone or thermopaste containing 10% taxol had only modest effects on tumor growth; tumor weights were reduced by only 10% and 35% respectively (Figure 21A). Therefore, thermopaste containing 20% taxol was more effective in reducing tumor growth than thermopaste containing 10% taxol (see Figure 21C; see also Figure 21B).

[0168] Thermopaste was detected in some of the animals at the site of administration. Polymer varying in weight between 0.026 g to 0.078 g was detected in 8 of 15 mice. Every animal in the group containing 20% taxol-loaded thermopaste contained some residual polymer suggesting that it was less susceptible to dissolution. Histologically, the tumors treated with taxol-loaded thermopaste contained lower cellularity and more tissue necrosis than control tumors. The vasculature was reduced and endothelial cells were frequently seen to be arrested in cell division. The taxol-loaded thermopaste did not appear to affect the integrity or cellularity of the skin or tissues surrounding the tumor. Grossly, wound healing was unaffected.

[0169] From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

[0170] "Preferred embodiments of the present invention will now be present as numbered clauses"

1. A composition, comprising:

    (a) an anti-angiogenic factor; and
    (b) a polymeric carrier.

2. The composition of clause 1 wherein said anti-angiogenic factor is Anti-Invasive Factor.

3. The composition of clause wherein said anti-angiogenic factor is retinoic acid and derivatives thereof.

4. The composition of clause 1 wherein said anti-angiogenic factor is selected from the group consisting of Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1 and Plasminogen Activator Inhibitor-2.

5. A composition, comprising:

    (a) taxol; and
    (b) a polymeric carrier.

6. The composition according to any one of clauses 1 to 5 wherein said composition is formed into microspheres having an average size of between 0.1 and 200 μm.

7. The composition according to any one of causes 1 to 5 wherein said composition is formed into a film with a thickness of between 100 μm and 2 mm.

8. The composition according to any one of clauses 1 to 5 wherein said composition is liquid above 45°C, and solid or semi-solid at 37°C.

9. The composition according to any one of clause 1 to 5 wherein said polymeric carrier is poly(ethylene-vinyl acetate) crosslinked with 40% vinyl acetate.

10. The composition according to any one of cause 1 to 5 wherein said polymeric carrier is poly(lactic-co-glycolic acid).

11. The composition according to any one of clause 1 to 5 wherein said polymeric carrier is polycaprolactone.

12. The composition according to any one of clause 1 to 5 wherein said polymeric carrier is polylactic acid.

13. The composition according to any one of clause 1 to 5 wherein said polymeric carrier is a copolymer of poly (ethylene-vinyl acetate) crosslinked with 40% vinyl acetate, and polylactic acid.

14. The composition according to any one of clause 1 to 5 wherein said polymeric carrier is a copolymer of polylactic acid and polycaprolactone.

15. A method for embolizing a blood vessel, comprising delivering into said vessel a therapeutically effective amount of composition according to any one of clauses 1-14, such that said blood vessel is effectively occluded.

16. The method clause 13 wherein said blood vessel nourishes a tumor.

17. A stent, comprising a generally tubular structure, the surface of which is coated with a composition according to any one of clauses 1-14.

18. A method for expanding the lumen of a body passageway, comprising inserting a stent into the passageway, the stent having a generally tubular structure, the surface of said structure being coated with a composition according to any one of clauses 1-14, such that said passageway is expanded.

19. A method for eliminating vascular obstructions, comprising inserting a vascular stent into a vascular passageway, the stent having a generally tubular structure, the surface of said structure being coated with a composition according to any one of clauses1-14, such that said vascular obstruction is eliminated.

20. A method for eliminating biliary obstructions, comprising inserting a biliary stent into a biliary passageway, the stent having a generally tubular structure, the surface of said structure being coated with a composition according to any one of clauses 1-14, such that said biliary obstruction is eliminated.

21. A method for eliminating urethral obstructions, comprising inserting a urethral stent into a urethra, the stent having a generally tubular structure, the surface of said structure being coated with a composition according to any one of clauses 1-14, such that said urethral obstruction is eliminated.

22. A method for eliminating esophageal obstructions, comprising inserting an esophageal stent into an esophagus, the stent having a generally tubular structure, the surface of said structure being coated with a composition according to any one of classes 1-14, such that said esophageal obstruction is eliminated.

23. A method for eliminating tracheal/bronchial obstructions, comprising inserting a tracheal/bronchial stent into the trachea or bronchi, the stent having a generally tubular structure, the surface of which is coated with a composition according to any one of clauses1-14, such that said tracheal/bronchial obstruction is eliminated.

24. A. method for treating a tumor excision site, comprising administering a composition according to any one of clauses 1-14 to the resection margin of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at said site is inhibited.

25. The method according to clause 24 wherein said anti-angiogenic composition is a thermopaste.

26. The method according to clause 24 wherein the step of administering comprising spraying a composition of nanospheres comprised of an anti-angiogenic composition into the resection margin of the tumor.

27. A method for treating corneal neovascularization, comprising administering a therapeutically effective amount of a composition according to any one of clauses 1-14 to the cornea, such that the formation of blood vessels is inhibited.

28. The method of clause 23 wherein said composition further comprises a topical corticosteroid.

29. A method for inhibiting angiogenesis in patients with non-tumorigenic, angiogenesis-dependent diseases, comprising administering a therapeutically effective amount of a composition comprising taxol to a patient with a non-tumorigenic angiogenesis-dependent disease, such that the formation of new blood vessels is inhibited.

30. A method for embolizing a blood vessel in a non-tumorigenic, angiogenesis-dependent diseases, comprising delivering to said vessel a therapeutically effective amount of a composition comprising taxol, such that said blood vessel is effectively occluded.

31. A method for expanding the lumen of a body passageway, comprising inserting a stent into the passageway, the stent having a generally tubular structure, the surface of said structure being coated with a composition comprising taxol, such that said passageway is expanded.

32. A method for eliminating vascular obstructions, comprising inserting a vascular stent into a vascular passageway, the stent having a generally tubular structure, the surface of said structure being coated with a composition comprising taxol, such that said vascular obstruction is eliminated.

33. A method for eliminating biliary obstructions, comprising inserting a biliary stent into a biliary passageway, the stent having a generally tubular structure, the surface of said structure being coated with a composition comprising taxol, such that said biliary obstruction is eliminated.

34. A method for eliminating urethral obstructions, comprising inserting a urethral stent into a urethra, the stent having a generally tubular structure, the surface of said structure being coated with a composition comprising taxol, such that said urethral obstruction is eliminated.

35. A method for eliminating esophageal obstructions, comprising inserting an esophageal stent into an esophagus, the stent having a generally tubular structure, the surface of said structure being coated with a composition comprising taxol, such that said esophageal obstruction is eliminated.

36. A method for eliminating tracheal/bronchial obstructions, comprising inserting a tracheal/bronchial stent into the trachea or bronchi, the stent having a generally tubular structure, the surface of said structure being coated with a composition comprising taxol, such that said tracheal/bronchial obstruction is eliminated.

37. A method for treating a tumor excision site, comprising administering a composition comprising taxol to the resection margin of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at said site is inhibited.

38. A method for treating corneal neovascularization, comprising administering a therapeutically effective amount of a composition comprising taxol to the cornea, such that the formation of new vessels is inhibited.

39. A pharmaceutical product, comprising:

(a) taxol, in a container, and
(b) a notice associated with said container in form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by said agency of said taxol, for human or veterinary administration to treat non-tumorigenic angiogenesis-dependent diseases.

SEQUENCE LISTING

[0171]

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Arsenault, A. Larry
Burt, Helen M.

(ii) TITLE OF INVENTION: Anti-Angiogenic Compositions and Methods of Use

(iii) NUMBER OF SEQUENCES: 1

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(v) COMPUTER READABLE FORM:

    (A) MEDIUM TYPE: Floppy disk
    (B) COMPUTER: IBM PC compatible
    (C) OPERATING SYSTEM: PC-DOS/MS-DOS
    (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

    (A) APPLICATION NUMBER:
    (B) FILING DATE:
    (C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

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    (C) TELEX: 3723836

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide
    (v) FRAGMENT TYPE: N-terminal
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Cys Asp Pro Gly Tyr Ile Gly Ser Arg
1               5
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Claims

1. A method of manufacturing a stent, which method comprises affixing to the stent a composition comprising taxol or an analogue or derivative of taxol and a polymeric carrier, wherein the taxol or an analogue or derivative of taxol is anti-angiogenic by the CAM assay.

2. A method according to Claim 1 wherein the composition comprises a taxol analogue.

3. A method according to Claim 1 wherein the composition comprises taxol.

4. A method of manufacturing a stent according to any preceding claim wherein the stent is an esophageal stent, a vascular stent, a biliary stent, a pancreatic stent, a ureteric stent, a urethral stent, a lacrimal stent, a eustachian tube stent, a fallopian tube stent, a trachial stent, or a bronchial stent.

5. A method according to any preceding claim wherein the polymeric carrier is a non-biodegradable polymeric carrier.

6. A method according to any preceding claim wherein the polymeric carrier is a biodegradable polymeric carrier.

7. A method according to of any preceding claim wherein the polymeric carrier comprises poly (lactic-co-glycolic acid), polycaprolactone, polylactic acid, a copolymer of polylactic acid and polycaprolactone, polycaprolactone, polylactic acid, or gelatin.

8. A method according to any preceding claim wherein the composition further comprises at least one of an antibiotic, an anti-inflammatory, an anti- viral agent, an anti-fungal agent, an anti-protozoal agent, a hormone, a surfactant, a radioactive agent or a toxin.

9. A method according to any preceding claim wherein the composition is anti-angiogenic by the CAM assay.

Patentansprüche

1. Verfahren zur Herstellungeines Stents, wobei das Verfahren das Befestigen einer Zusammensetzung am Stent umfasst, die Taxol oder ein Taxol-Analogon oder -Derivat und einen polymeren Träger umfasst, wobei das Taxol oder Taxol-Analogon oder -Derivat im CAM-Test antiangiogen ist.

2. Verfahren nach Anspruch 1, wobei die Zusammensetzung ein Taxol-Analogon umfasst.

3. Verfahren nach Anspruch 1, wobei die Zusammensetzung Taxol umfasst.

4. Verfahren zur Herstellung eines Stents nach einem der vorhergehenden Ansprüche, wobei der Stent ein Speise- röhren-Stent, ein Gefäß-Stent, ein Gallen-Stent, ein Bauchspeicheldrüsen-Stent, ein Harnleiter-Stent, ein Harnröh- ren-Stent, ein Tränendrüsen-Stent, ein Ohrtrompeten-Stent, ein Eileiter-Stent, ein Luftröhren-Stent oder ein Bron- chial-Stent ist.

5. Verfahren nach einem der vorhergehenden Ansprüche, wobei der polymere Träger ein biologisch nicht abbaubarer polymerer Träger ist.

6. Verfahren nach einem der vorhergehenden Ansprüche, wobei der polymere Träger ein biologisch abbaubarer po- lymerer Träger ist.

7. Verfahren nach einem der vorhergehenden Ansprüche, wobei der polymere Träger Polymilchsäure-co-glycolsäure, Polycaprolacton, Polymilchsäure, ein Copolymer aus Polymilchsäure und Polycaprolacton, Polycaprolacton, Poly- milchsäure, oder Gelatine umfasst.

8. Verfahren nach einem der vorhergehenden Ansprüche, wobei die Zusammensetzung ferner eines der Folgenden umfasst: ein Antibiotikum, einen Entzündungshemmer, ein Antivirusmittel, ein Antipilzmittel, ein Antiprotozoenmittel, ein Hormon, ein Tensid, ein radiaktives Mittel oder ein Toxin.

9. Verfahren nach einem der vorhergehenden Ansprüche, wobei die Zusammensetzung im CAM-Test antiangiogen ist.

Revendications

1. Une méthode de fabrication d'une endoprothèse, cette méthode supposant l'ajout à l'endoprothèse d'une compo- sition contenant du taxol ou un analogue ou un dérivé du taxol et un vecteur polymérique, dans lequel le taxol ou un analogue ou un dérivé du taxol est anti-angiogenèse conformément au test de la membrane CAM.

2. Une méthode conformément à la revendication 1, dans laquelle la composition contient un analogue du taxol.

3. Une méthode conformément à la revendication 1, dans laquelle la composition contient du taxol.

4. Une méthode de fabrication d'une endoprothèse conformément à n'importe laquelle des revendications précédentes,

dans laquelle l'endoprothèse est une endoprothèse oesophagique, une endoprothèse vasculaire, une endoprothèse biliaire, une endoprothèse pancréatique, une endoprothèse urétérale, une endoprothèse urétrale, une endoprothèse lacrymale, une endoprothèse de la trompe auditive, une endoprothèse des trompes de Fallope, une endoprothèse trachéale ou une endoprothèse bronchique.

5. Une méthode conformément à n'importe laquelle des revendications précédentes, dans laquelle le vecteur polymérique est un vecteur polymérique non-biodégradable.

6. Une méthode conformément à n'importe laquelle des revendications précédentes, dans laquelle le vecteur polymérique est un vecteur polymérique biodégradable.

7. Une méthode conformément à n'importe laquelle des revendications précédentes, dans laquelle le vecteur polymérique comporte du poly (acide lactique-co-glycolique), du polycaprolactone, de l'acide polylactique, un copolymère de l'acide polylactique et du polycaprolactone, du polycaprolactone, de l'acide polylactique ou de la gélatine.

8. Une méthode conformément à n'importe laquelle des revendications précédentes, dans laquelle la composition comprend également au moins un antibiotique, un anti-inflammatoire, un agent antiviral, un agent antifongique, un agent antiprotozoaire, une hormone, un surfactant, un agent radioactif ou une toxine.

9. Une méthode conformément à n'importe laquelle des revendications précédentes, dans laquelle la composition est anti-angiogenèse conformément au test de la membrane CAM.
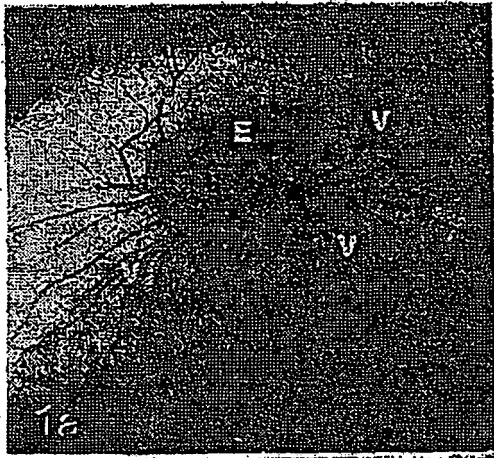
FIG. 1A

FIG. 1B
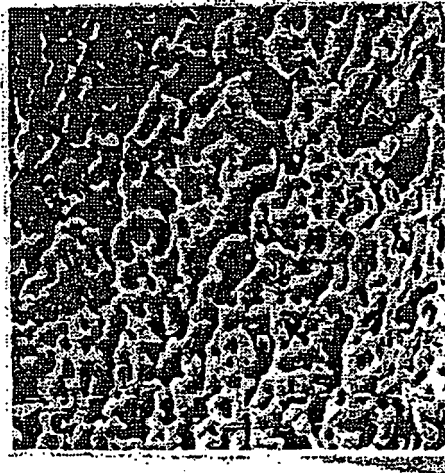
FIG. 1C

FIG. 1D

d
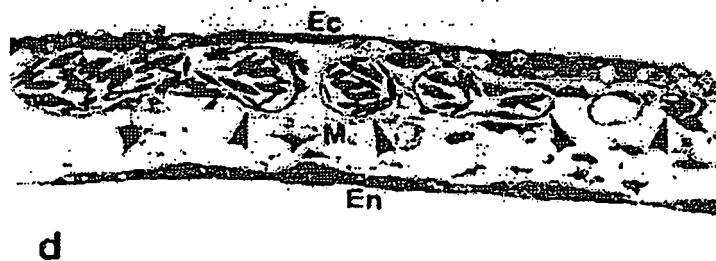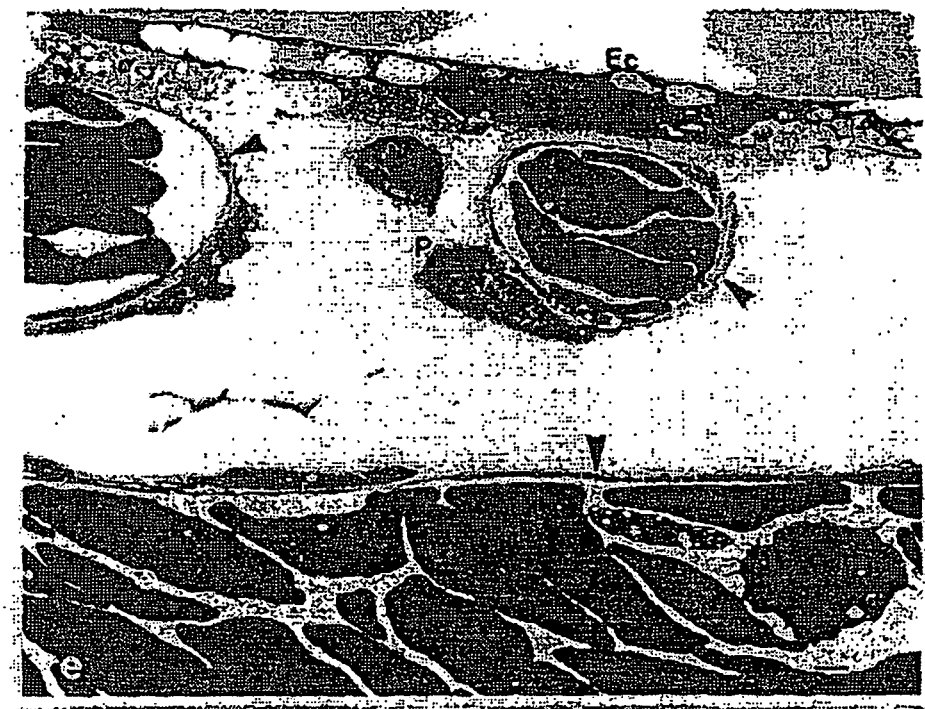
FIG. 1E

FIG. 2A

FIG. 2B

FIG. 2C

FIG. 2D
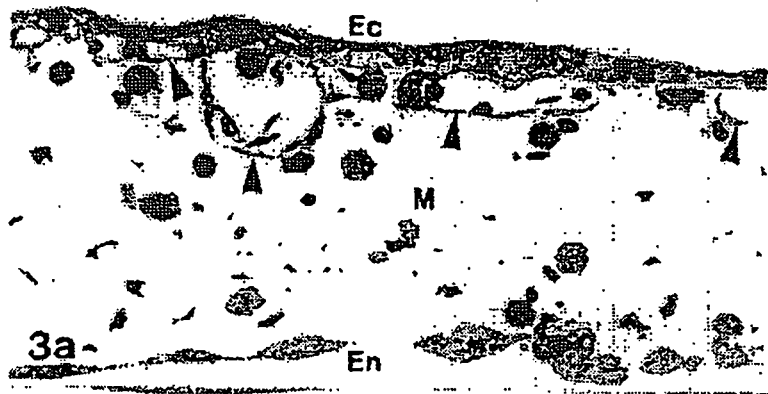
FIG. 3A

FIG. 3B

FIG. 3C

FIG. 4A

FIG. 4B

FIG. 4C

FIG. 5.

% OF SPHERES IN SIZE RANGE (BY NUMBER)

SIZE RANGE (μM DIAMETER)

FIG. 6.

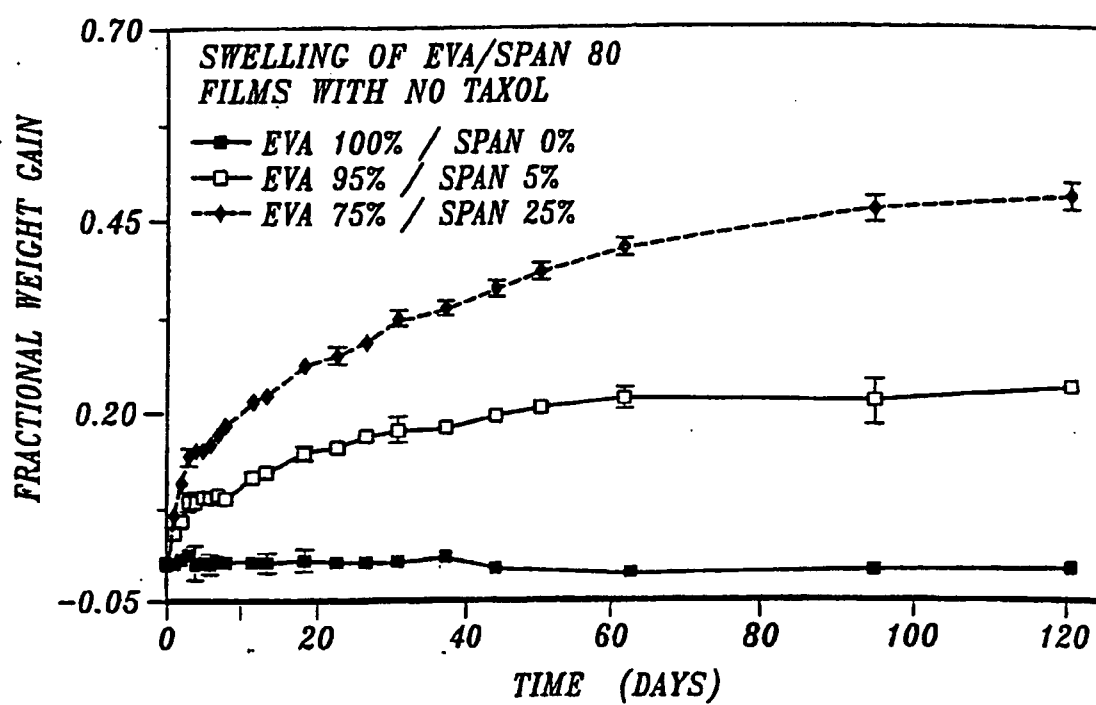*% OF SPHERES IN SIZE RANGE (BY WEIGHT)*
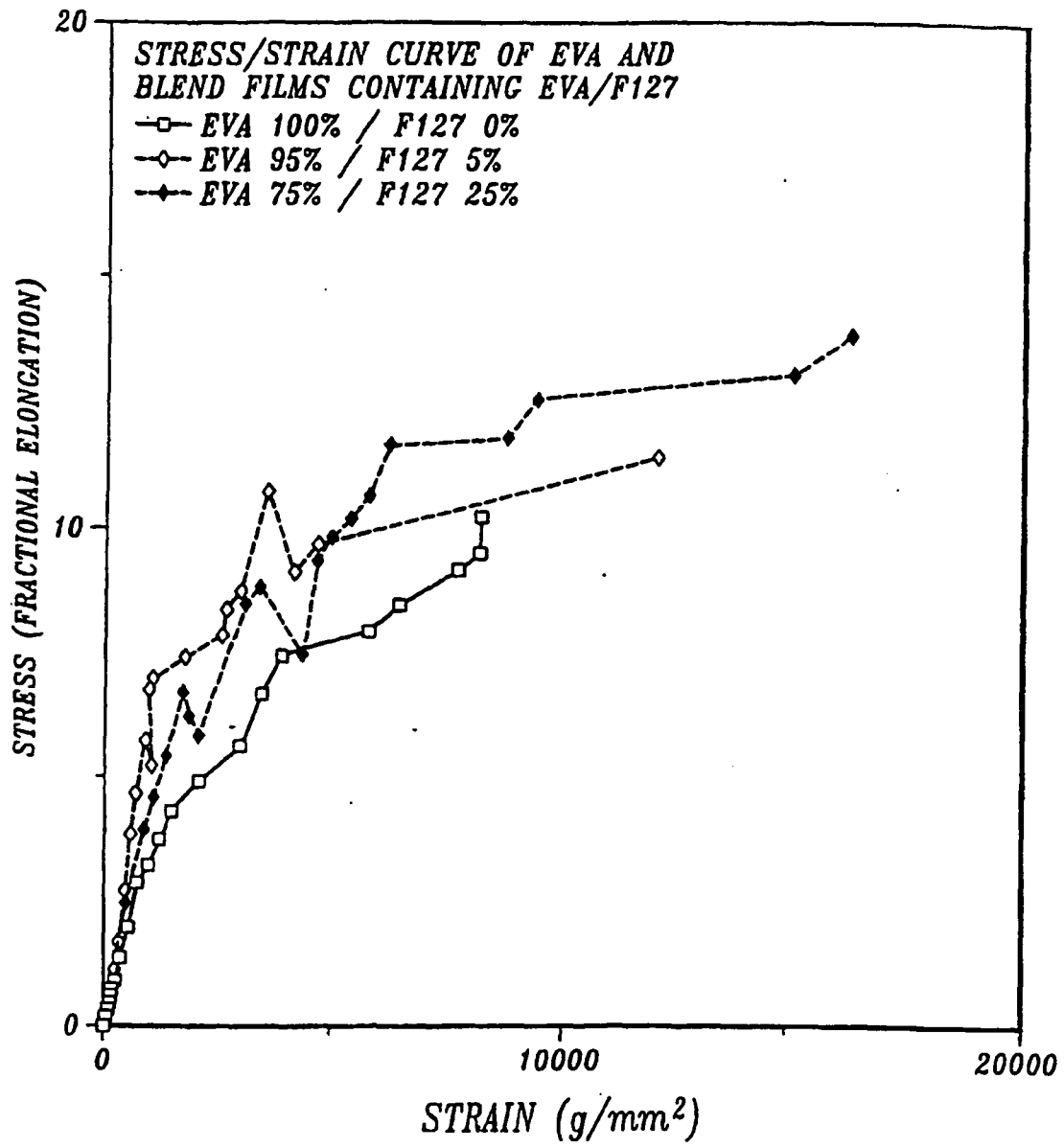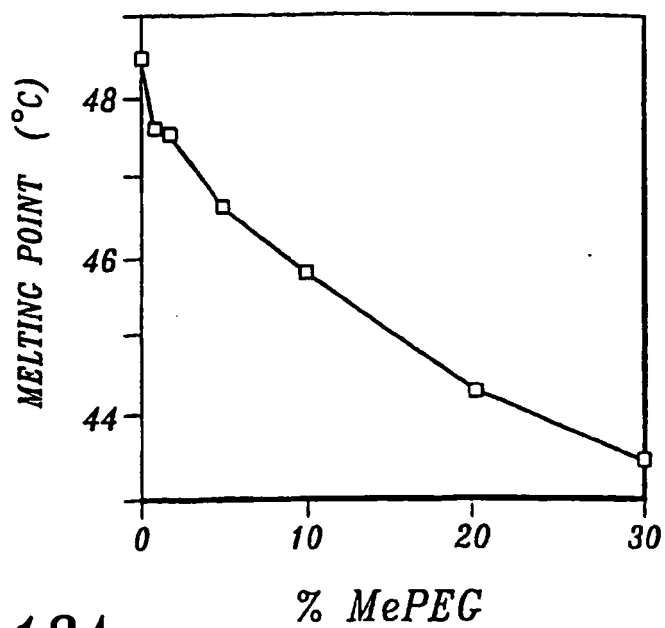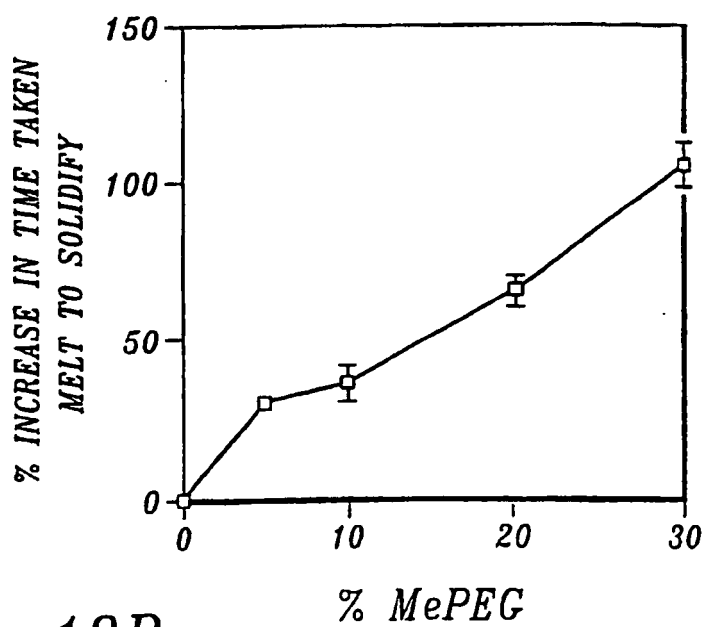
SIZE RANGE (μM DIAMETER)
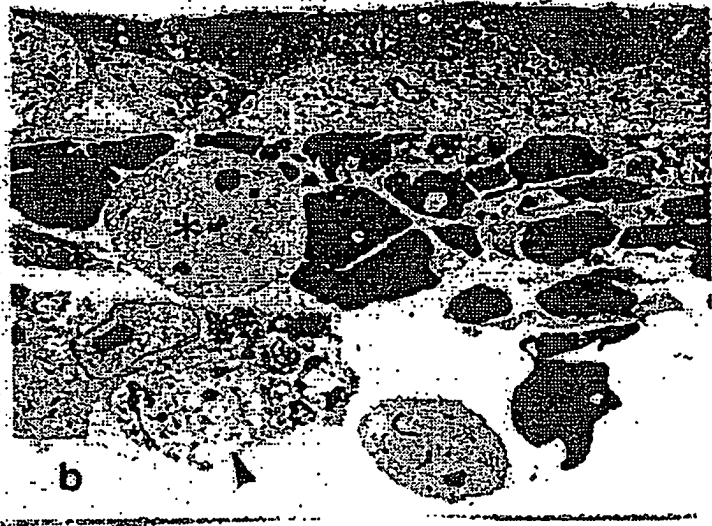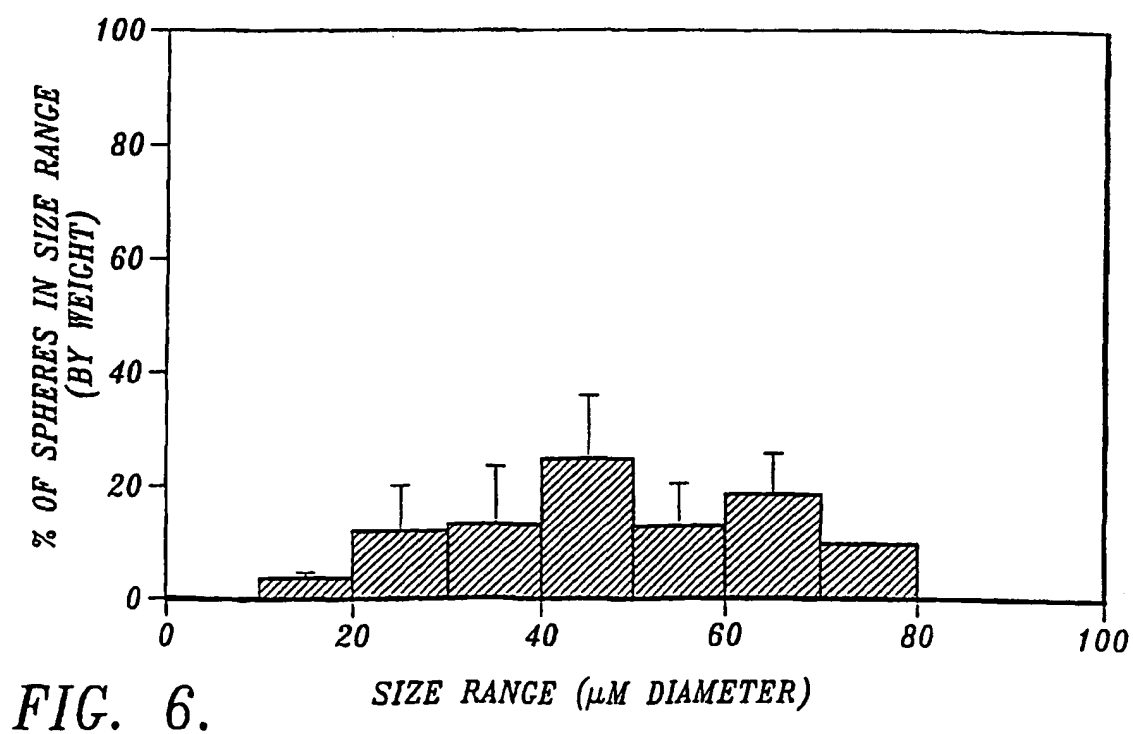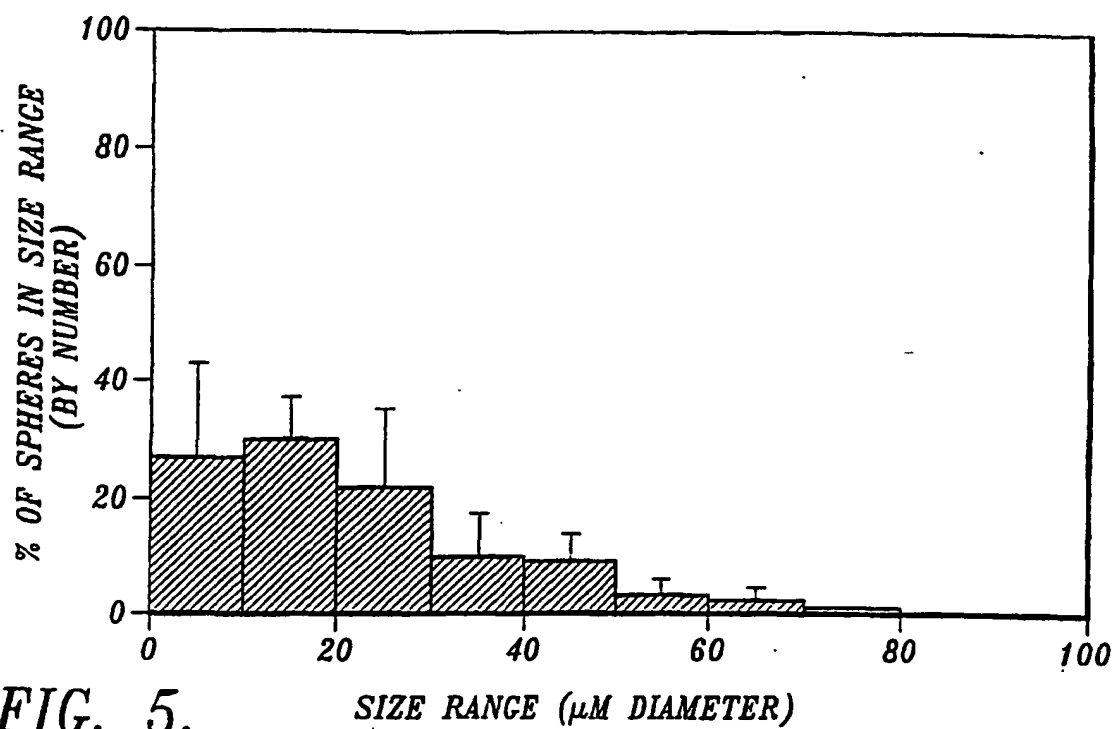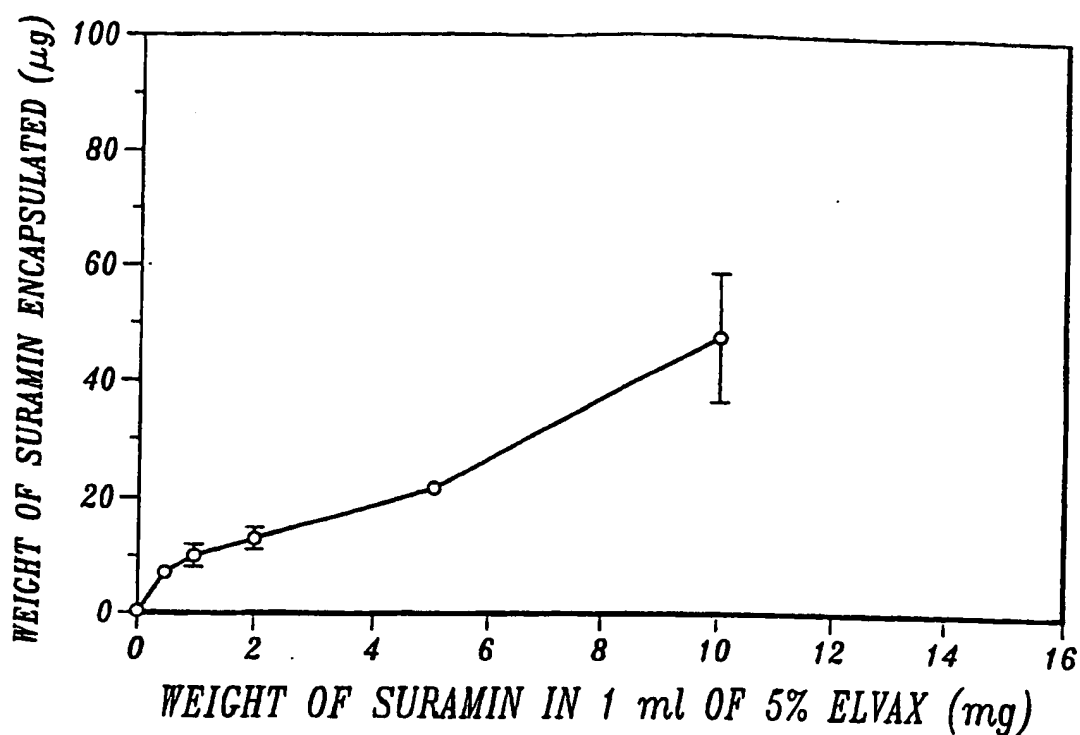
FIG. 7.

FIG. 8.

FIG. 9.

FIG. 10.

FIG. 11.

FIG. 12.

FIG. 13A.

FIG. 13B.
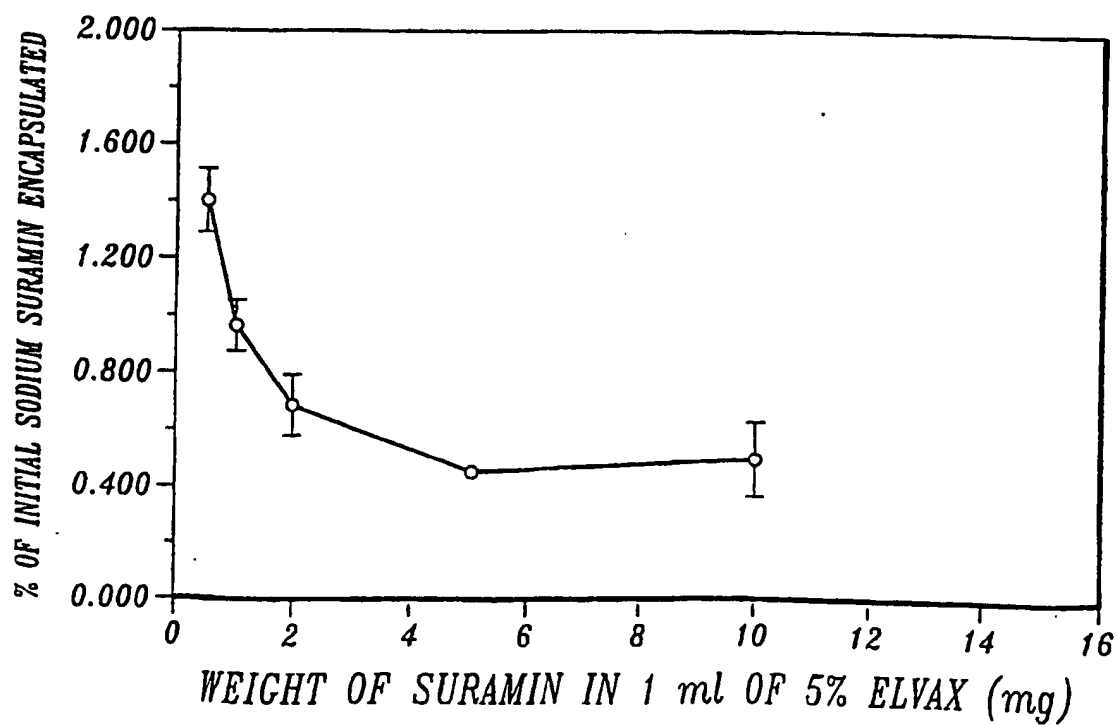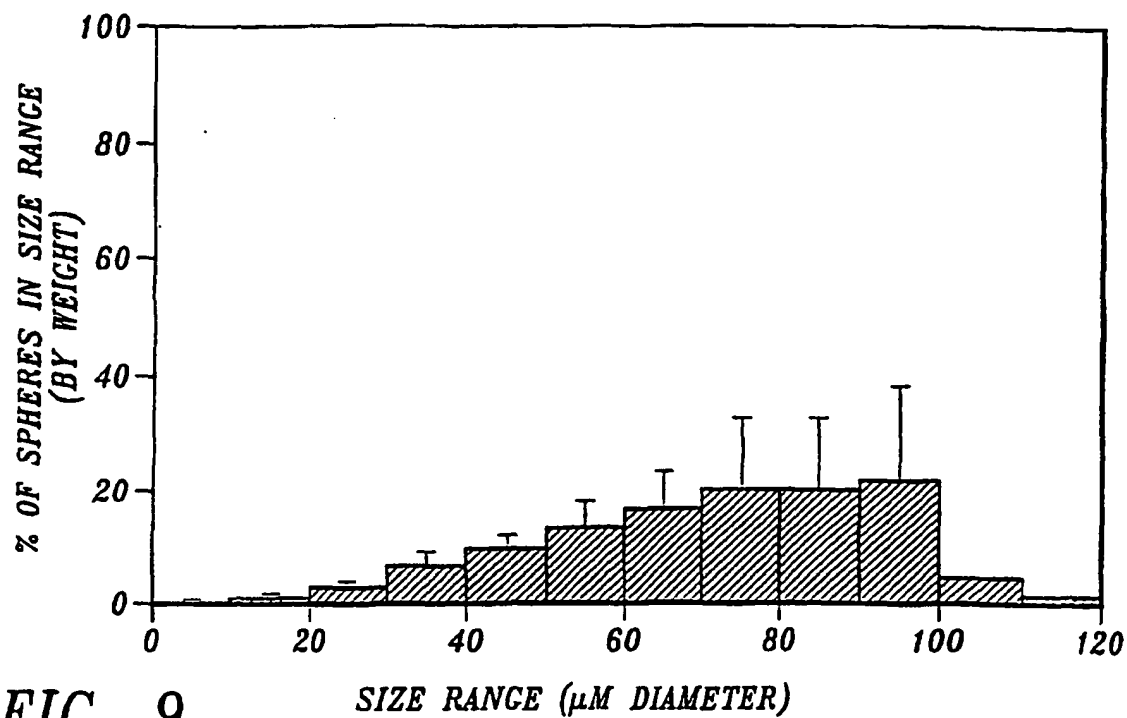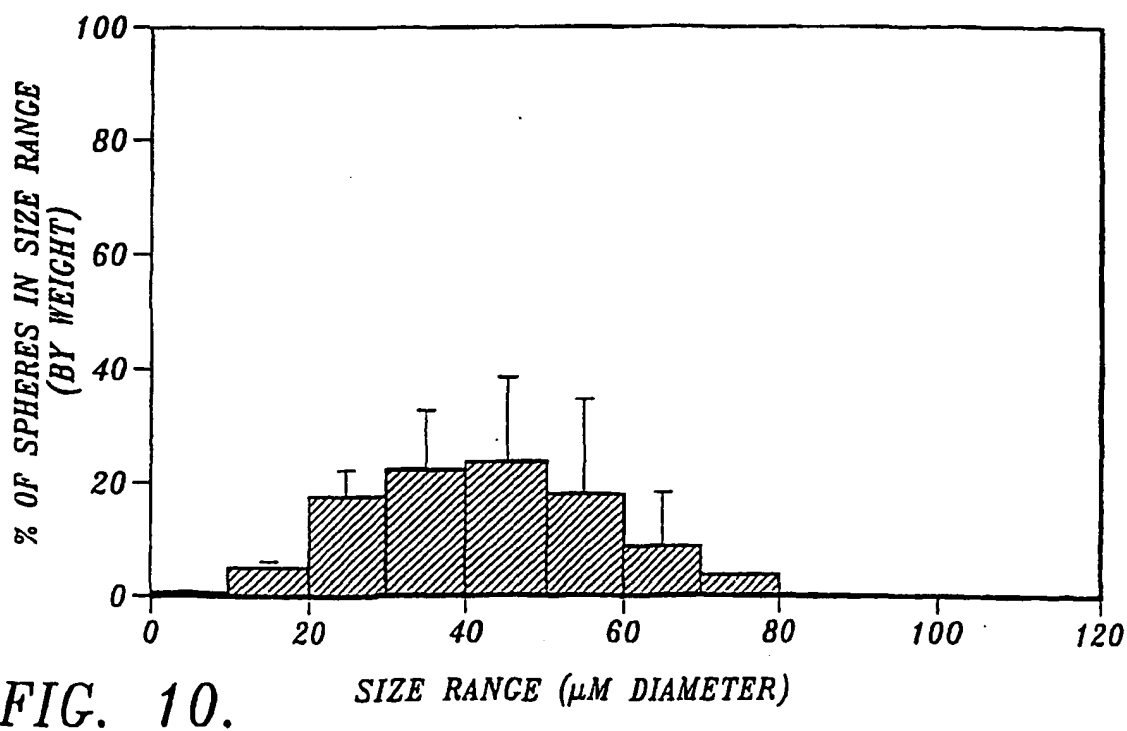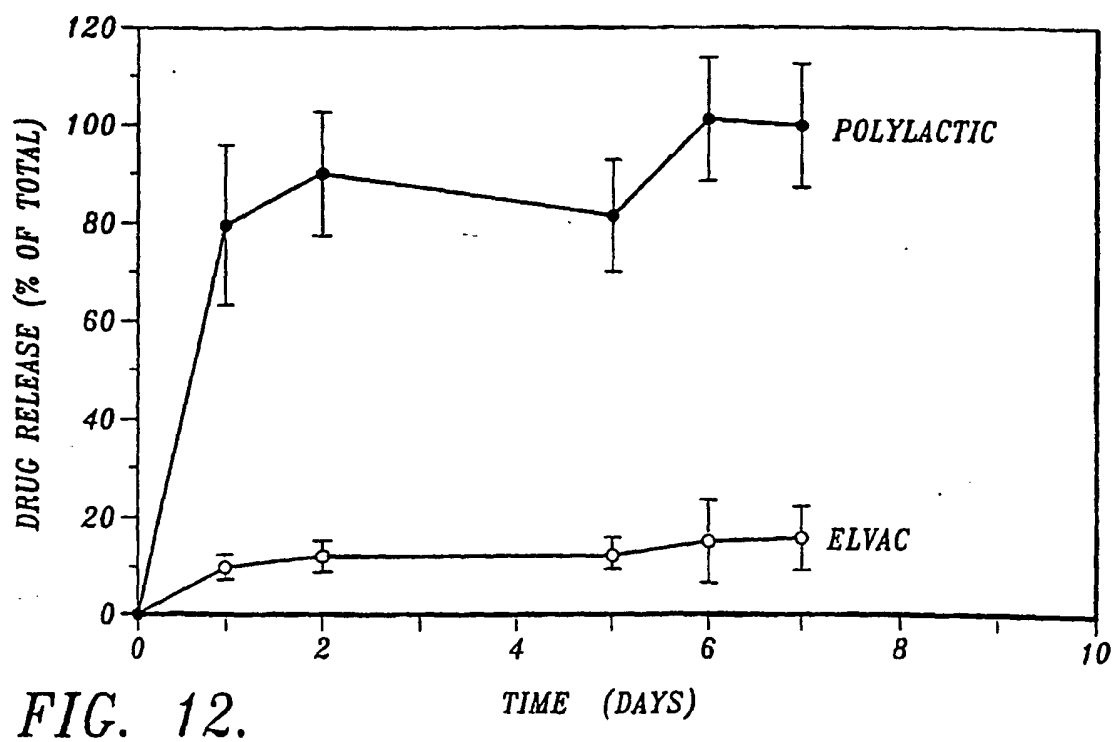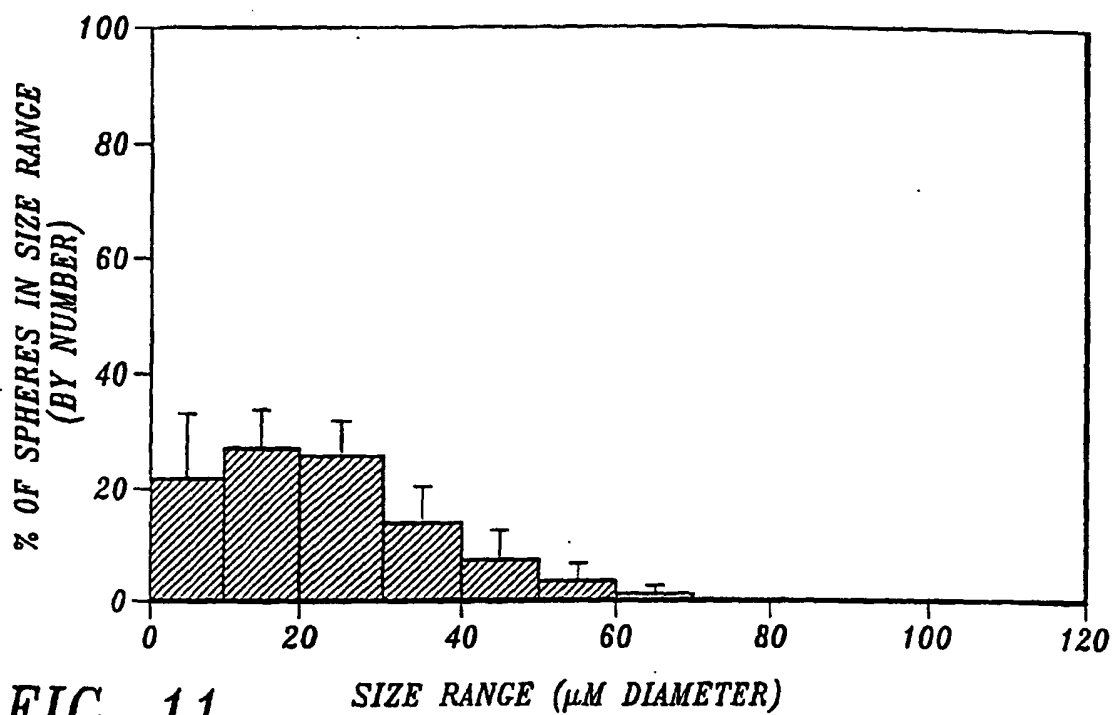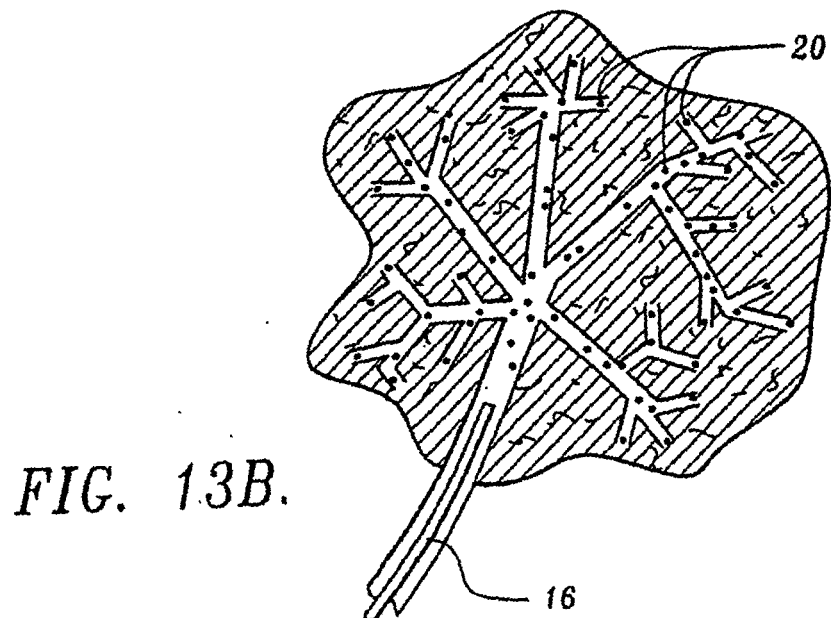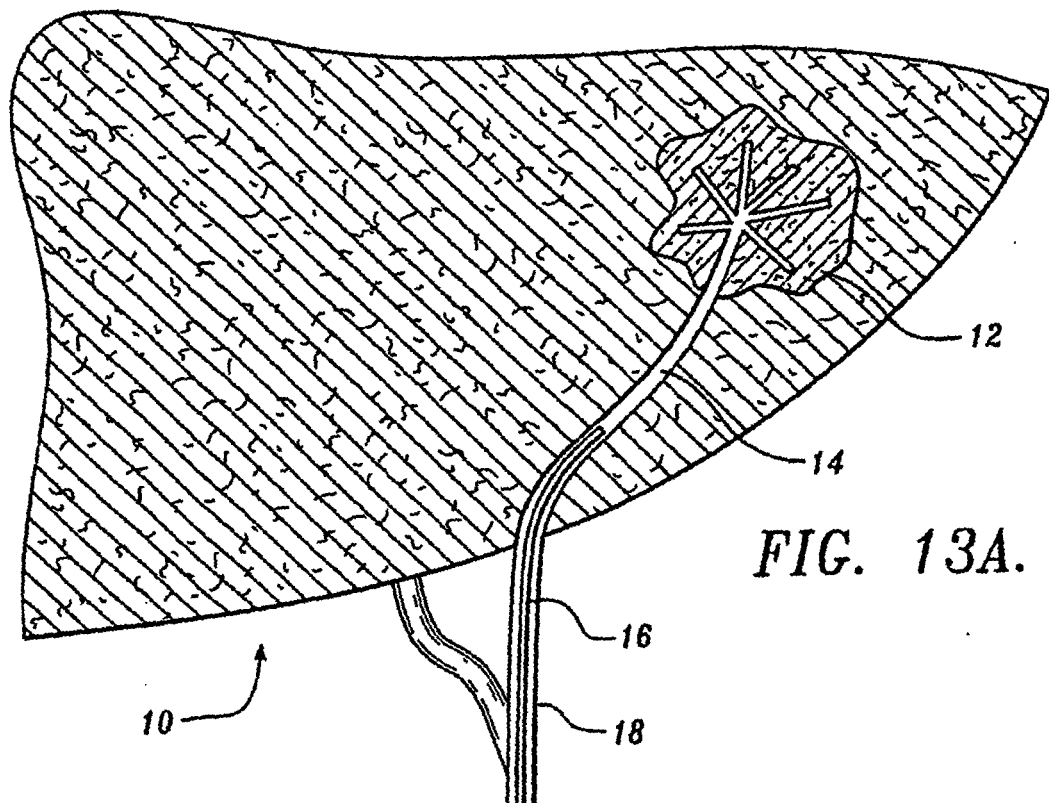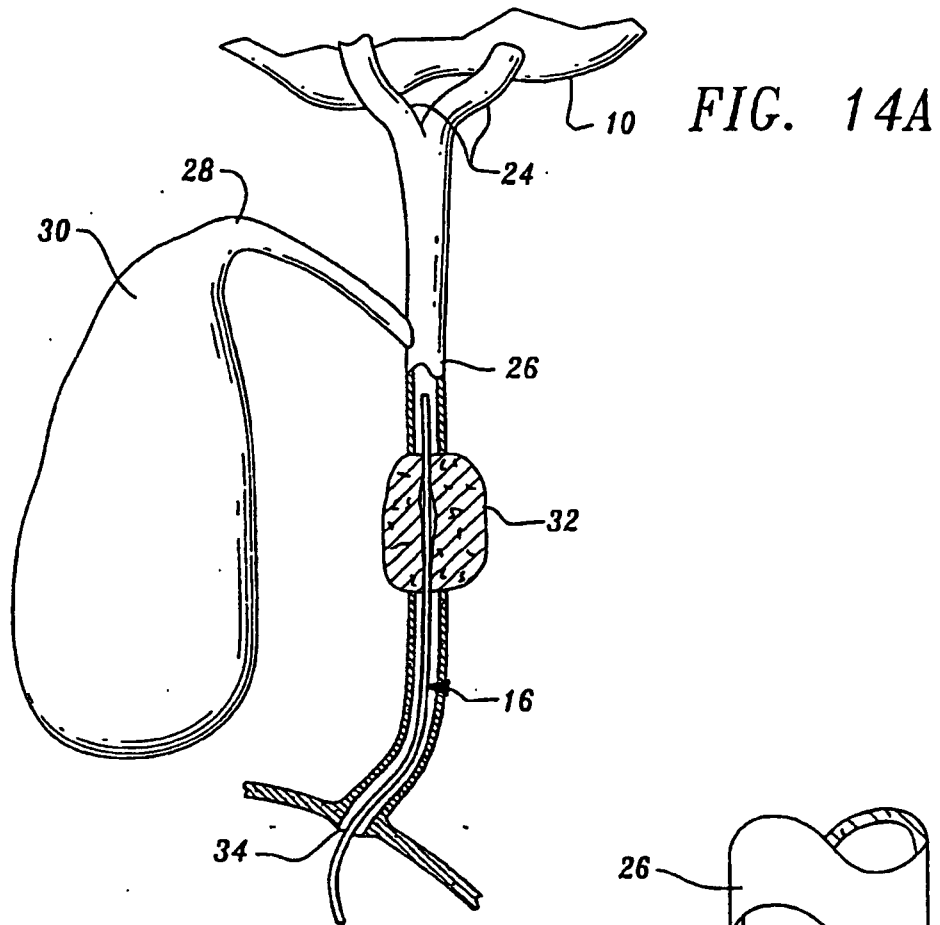
FIG. 14A

FIG. 14B
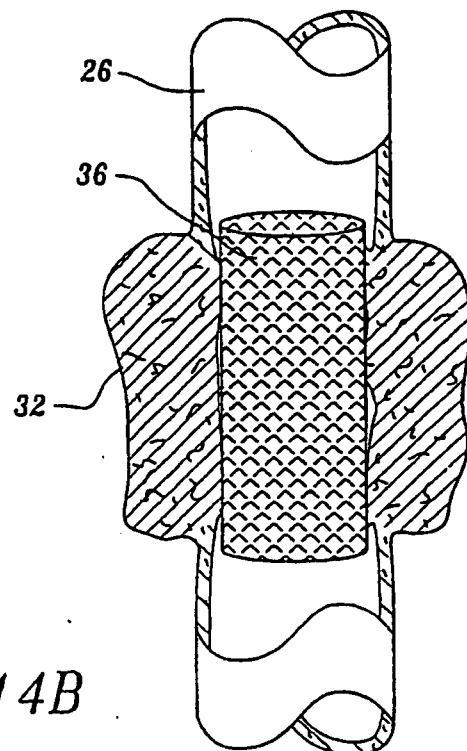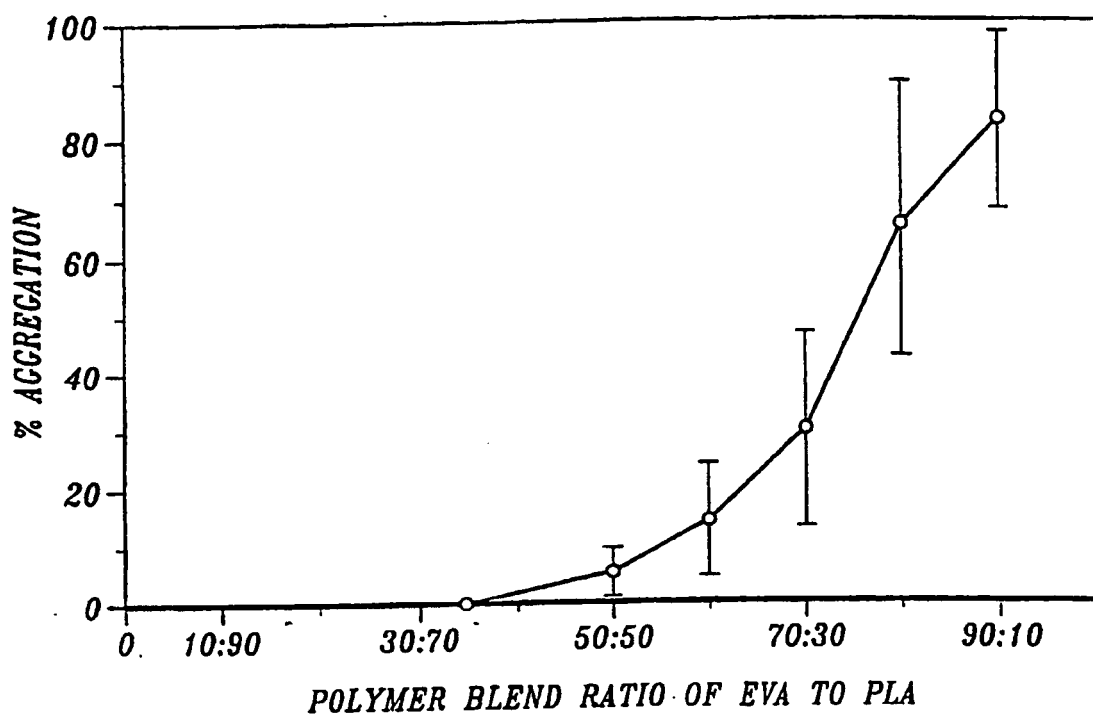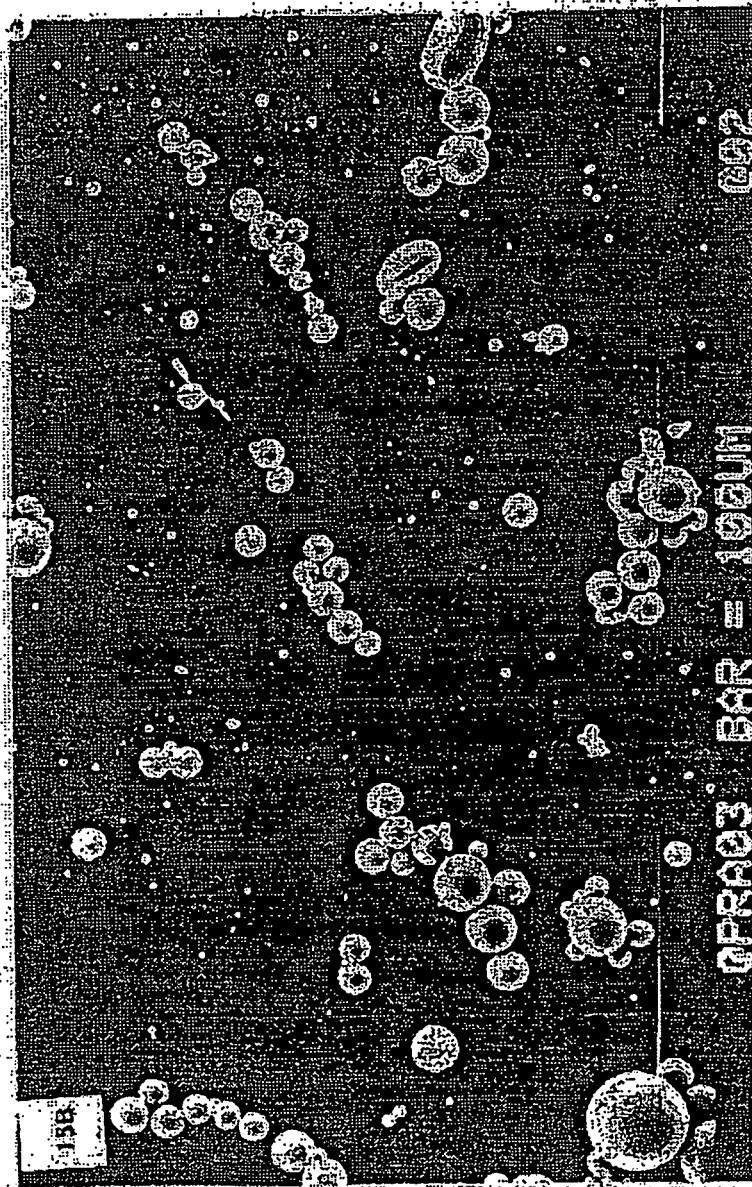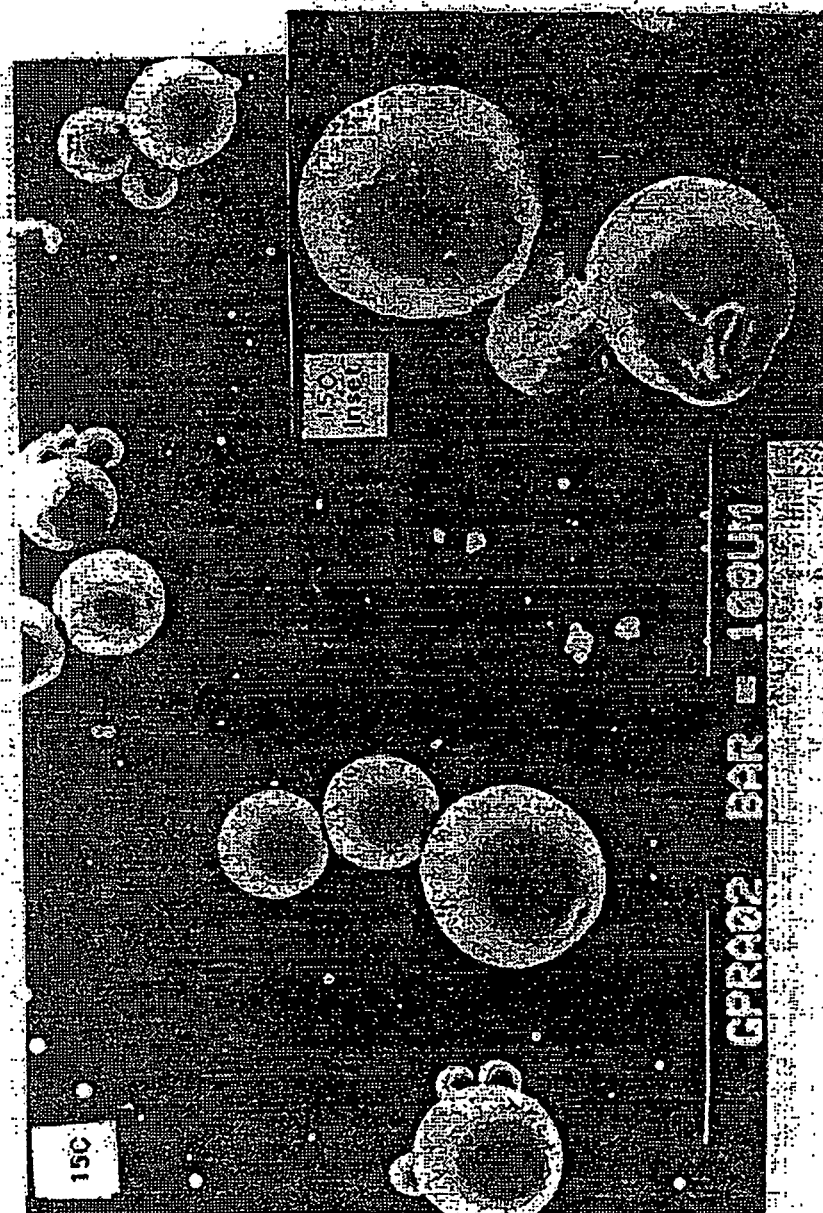
FIG. 15A.

FIG. 15B

FIG. 15C
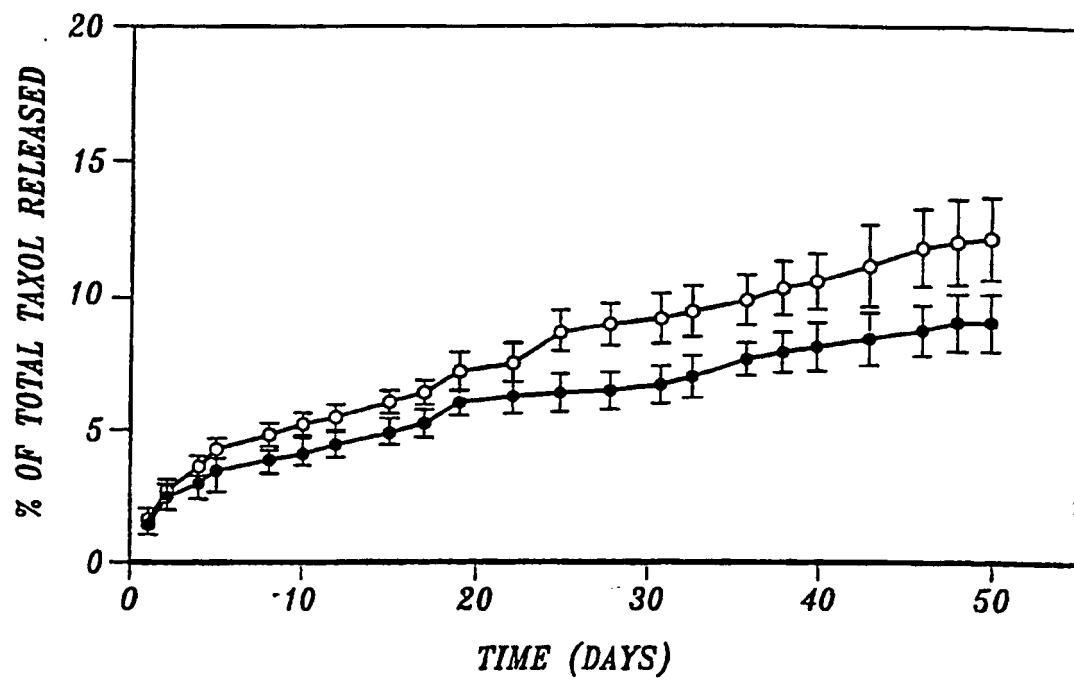
FIG. 15D.

FIG. 15E
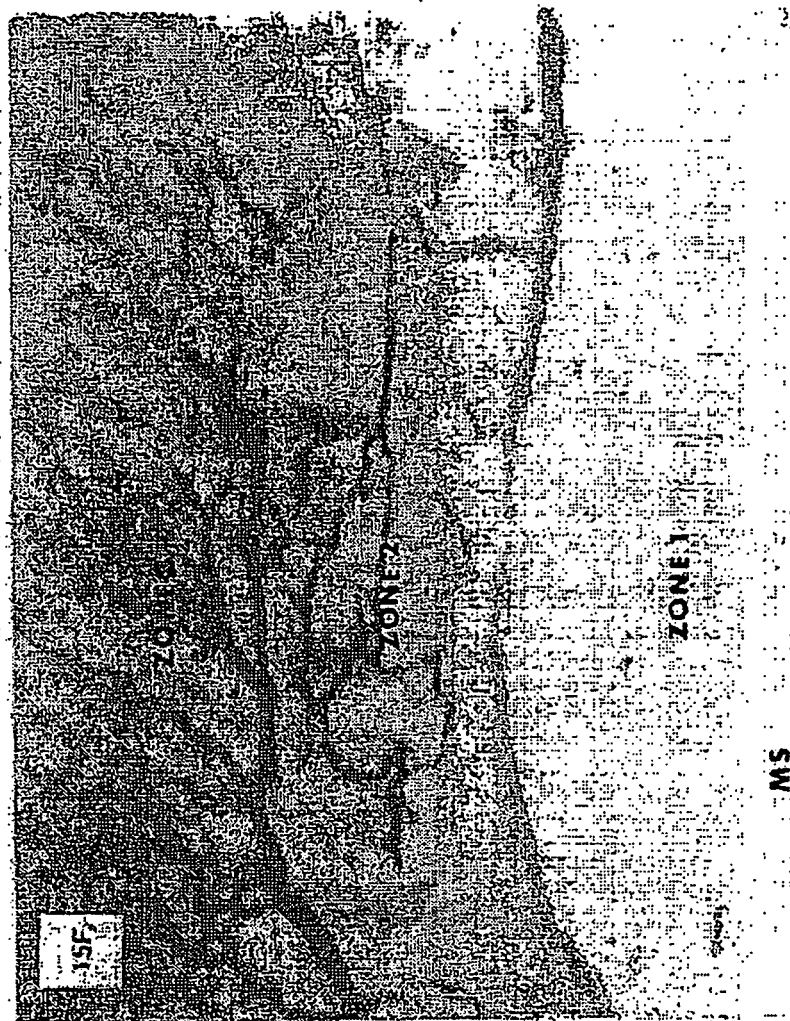
FIG. 15F
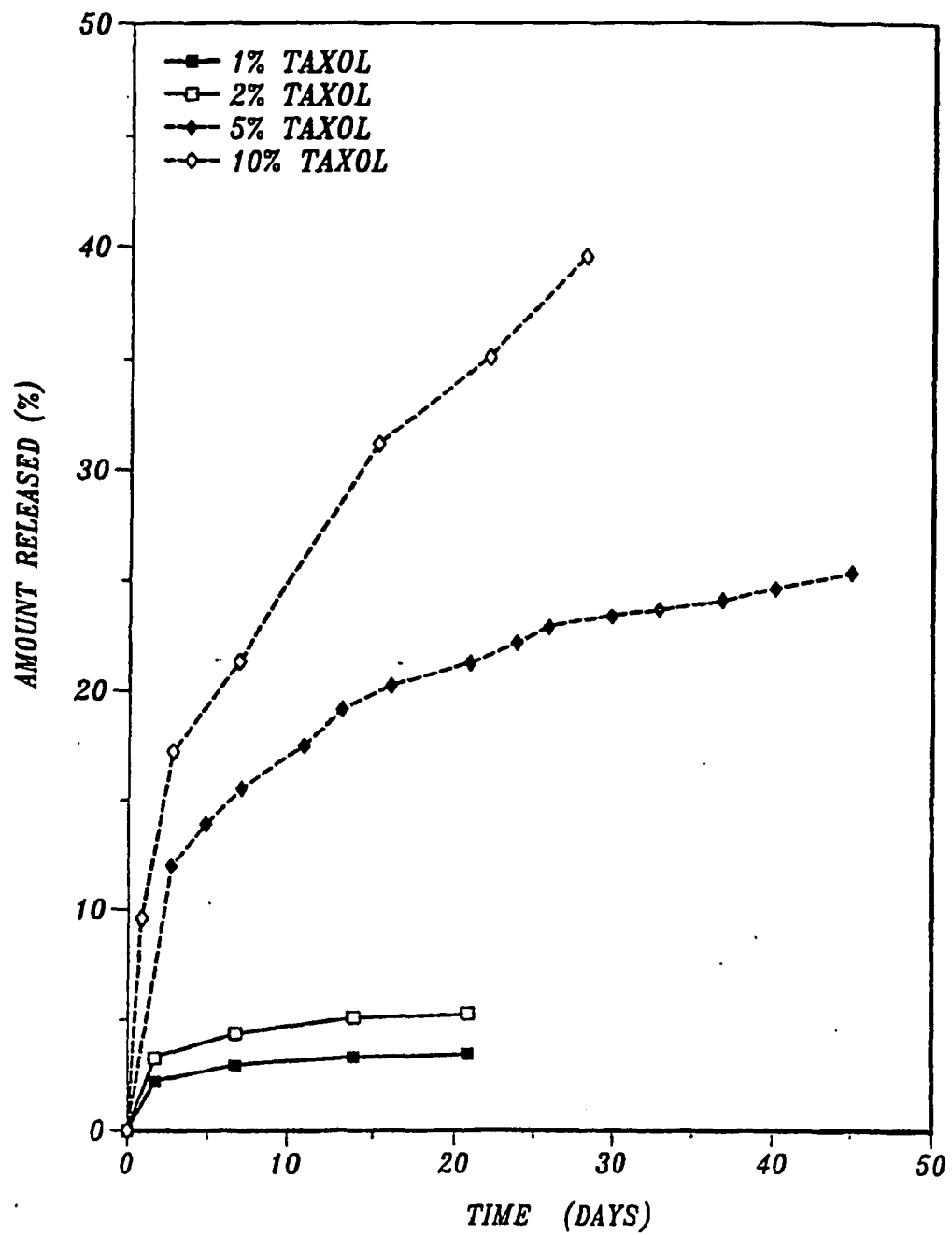
FIG. 16A.

FIG. 16B
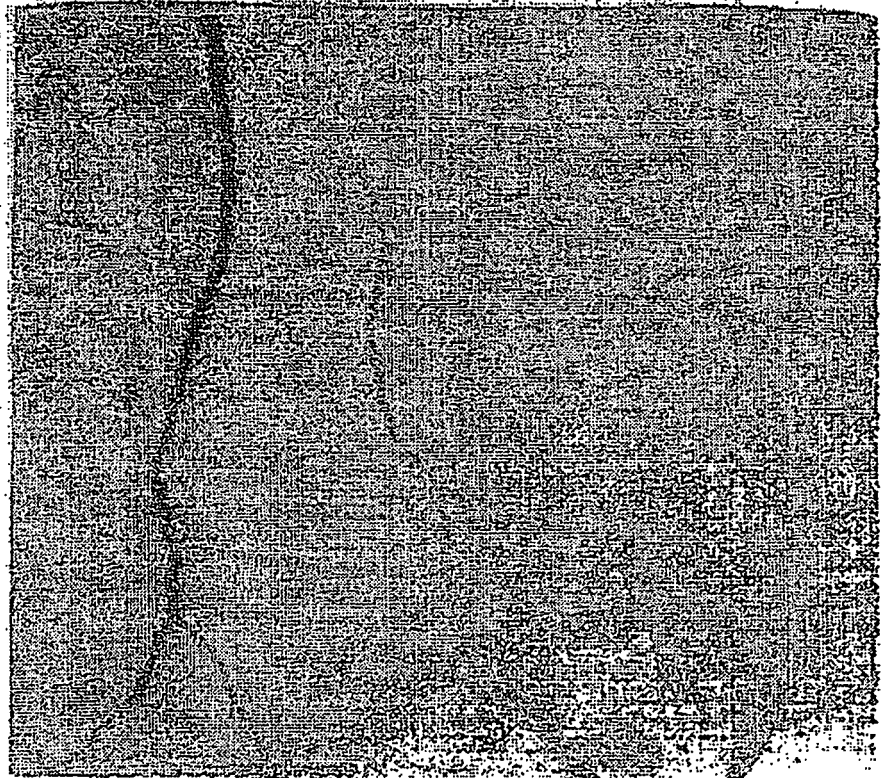
FIG. 16C
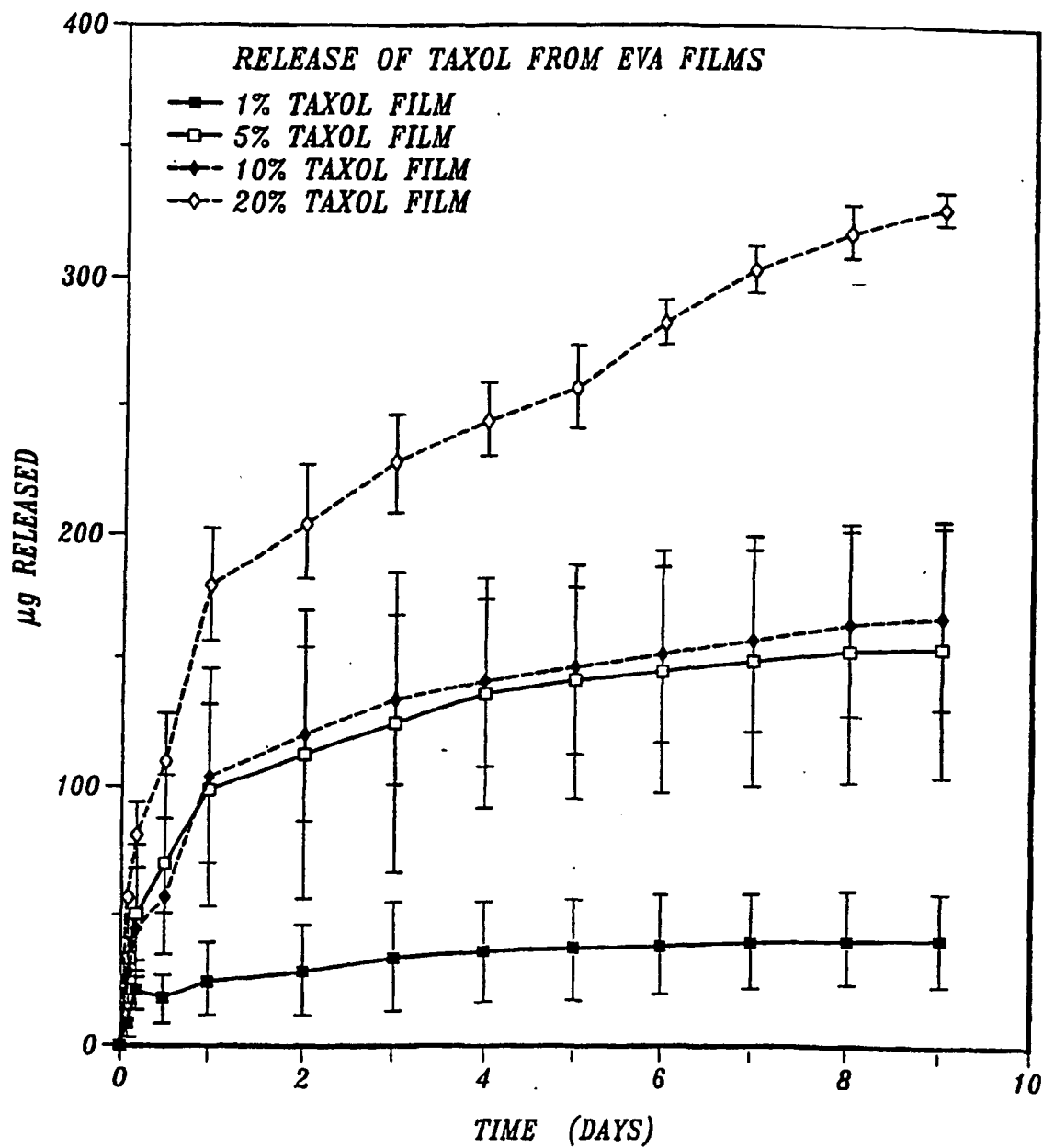
FIG. 17A.
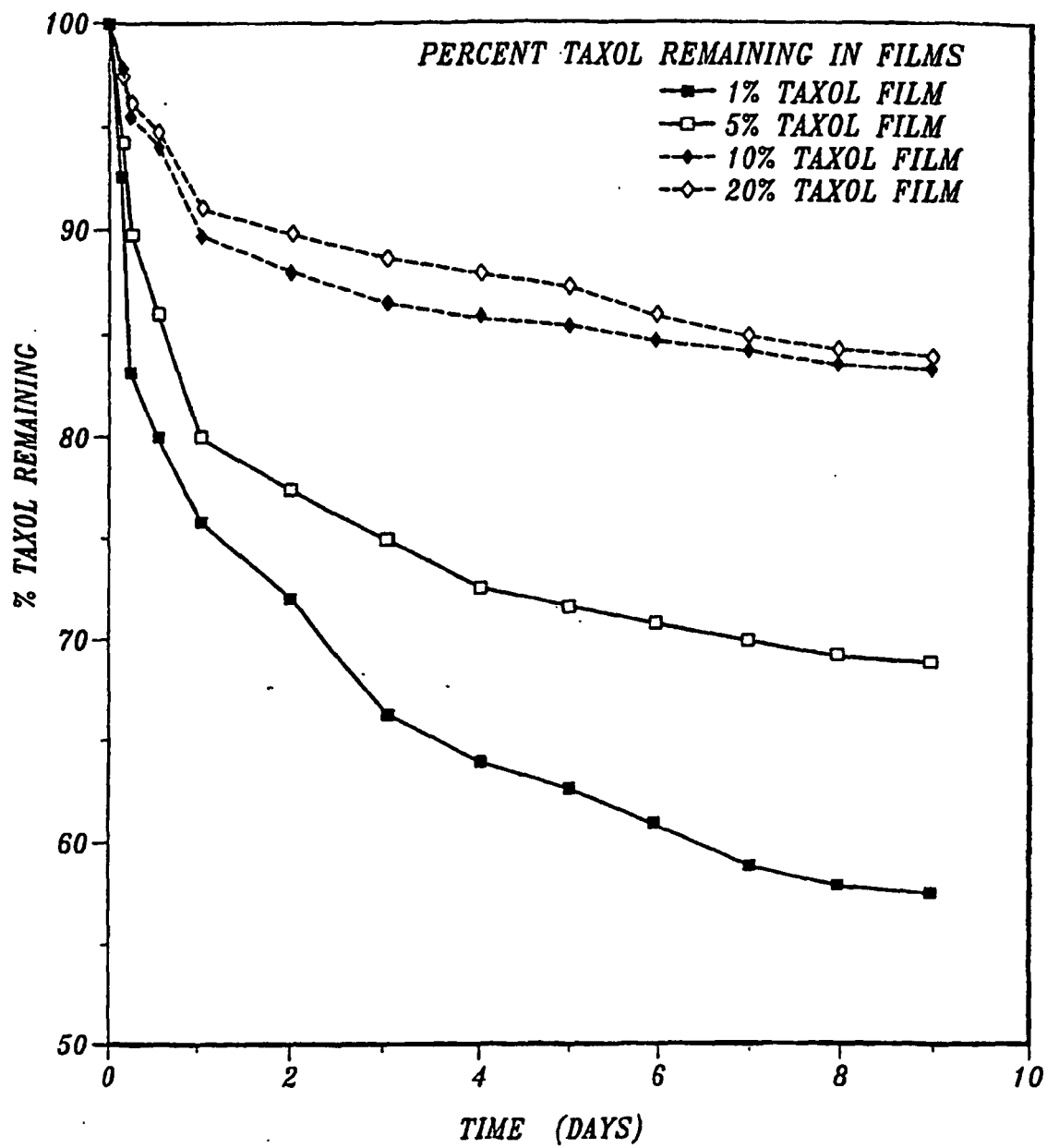
FIG. 17B.
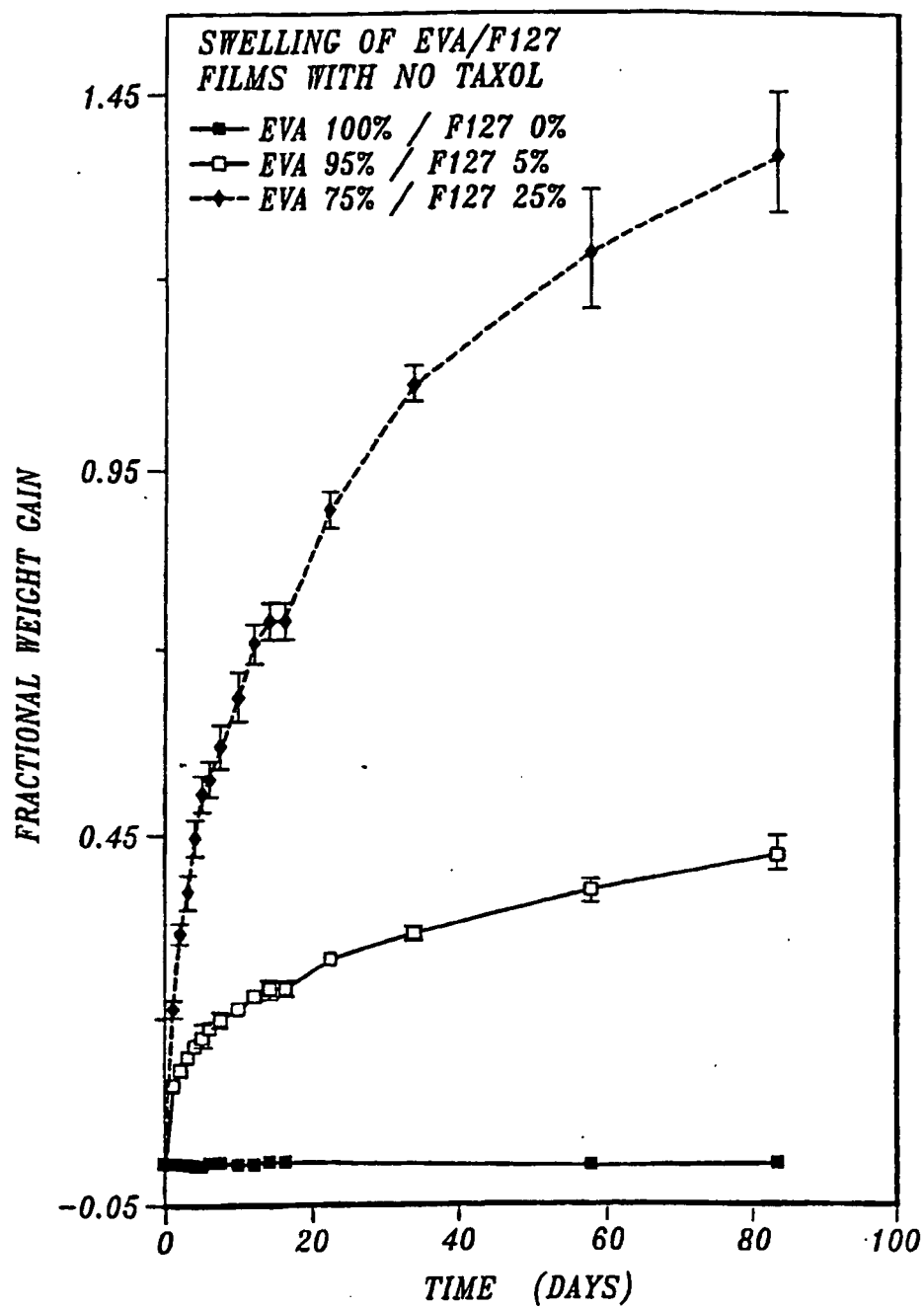
FIG. 17C.

FIG. 17D.

FIG. 17E.

FIG. 18A.

FIG. 18B.
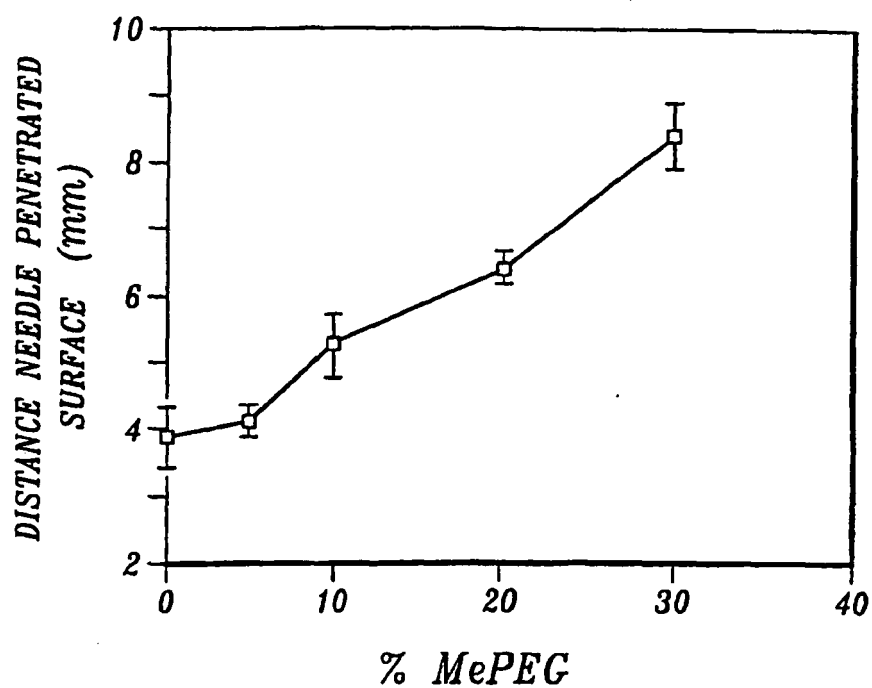
FIG. 18C.
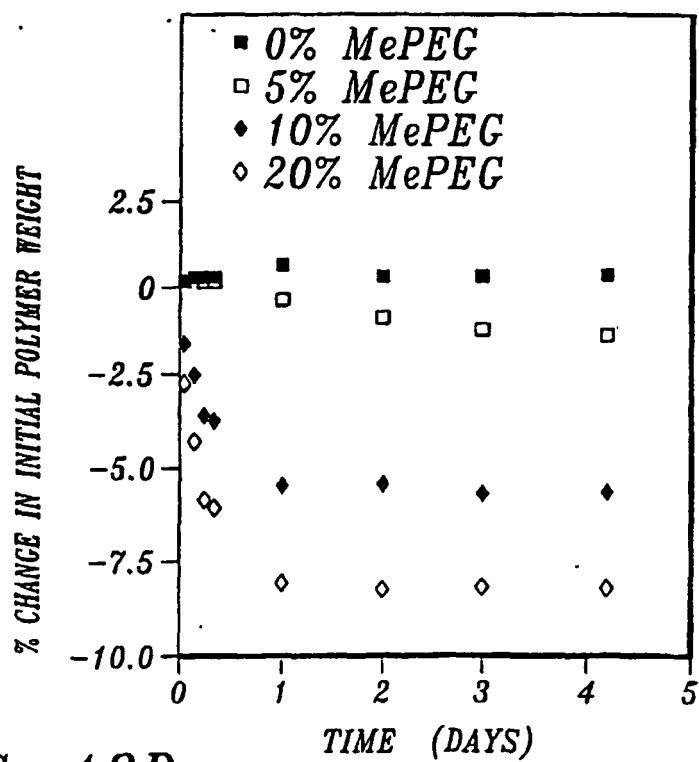
FIG. 18D.
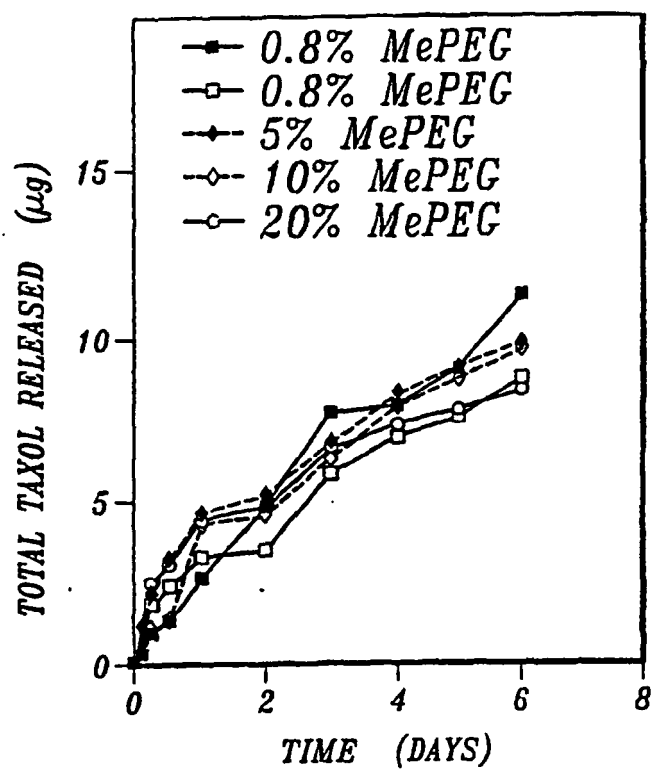
FIG. 18E.
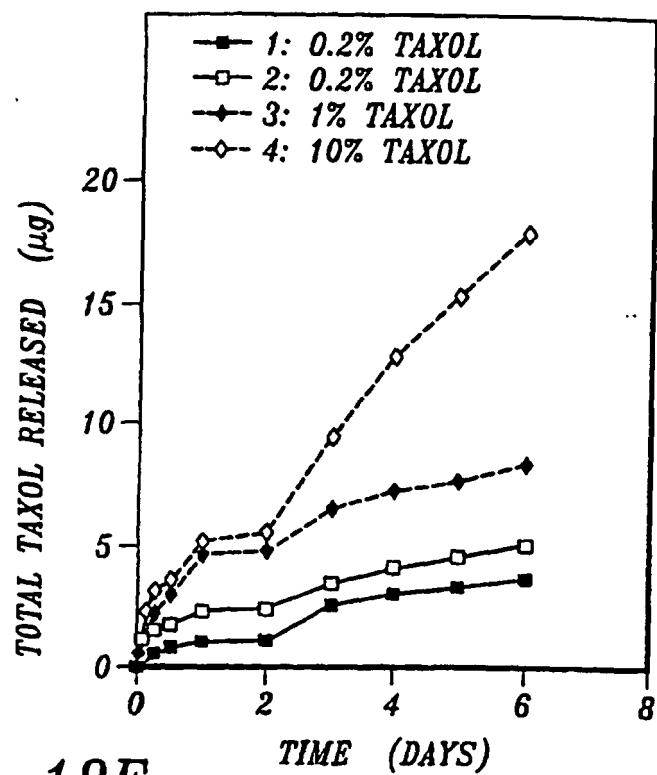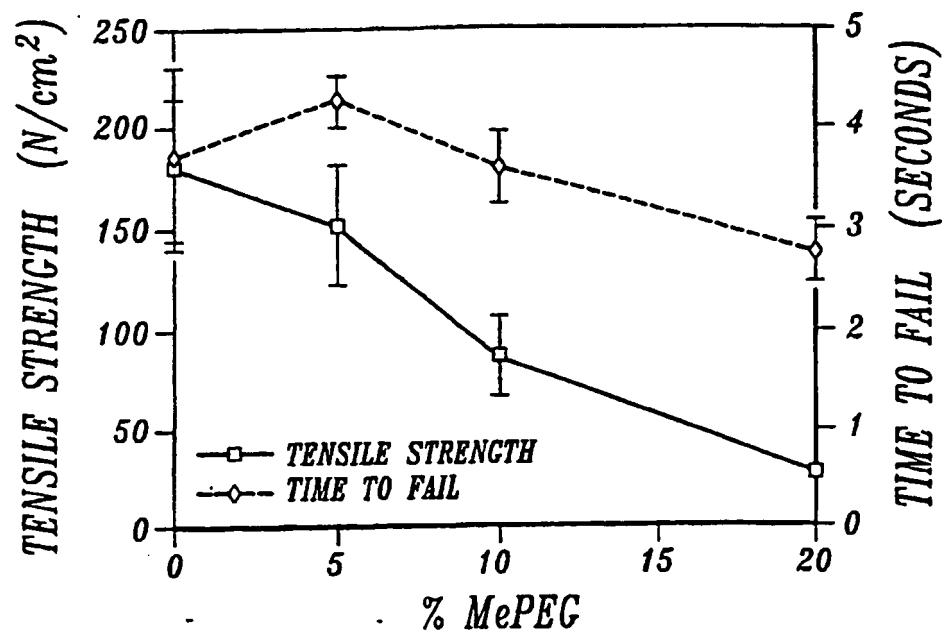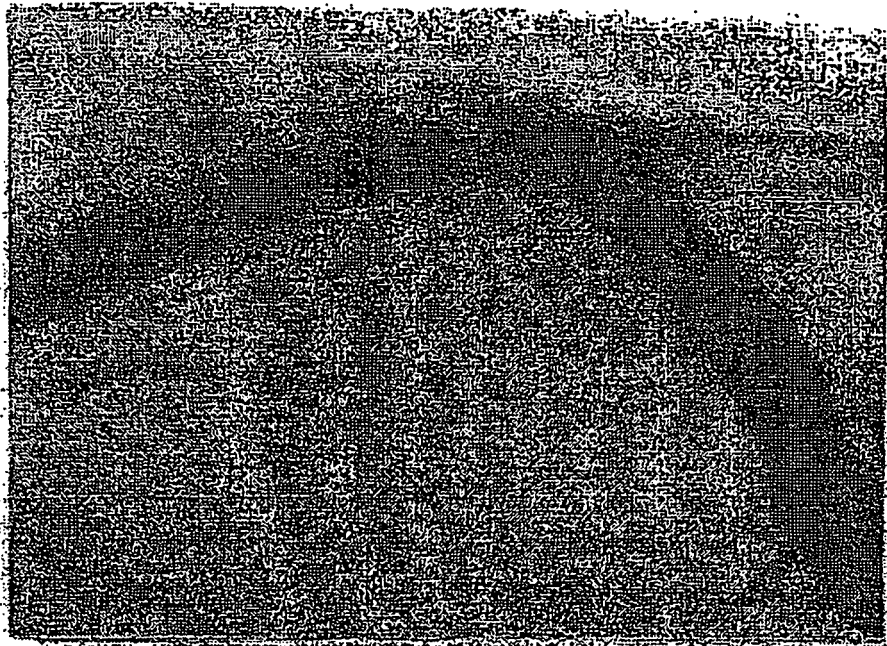
FIG. 18F.
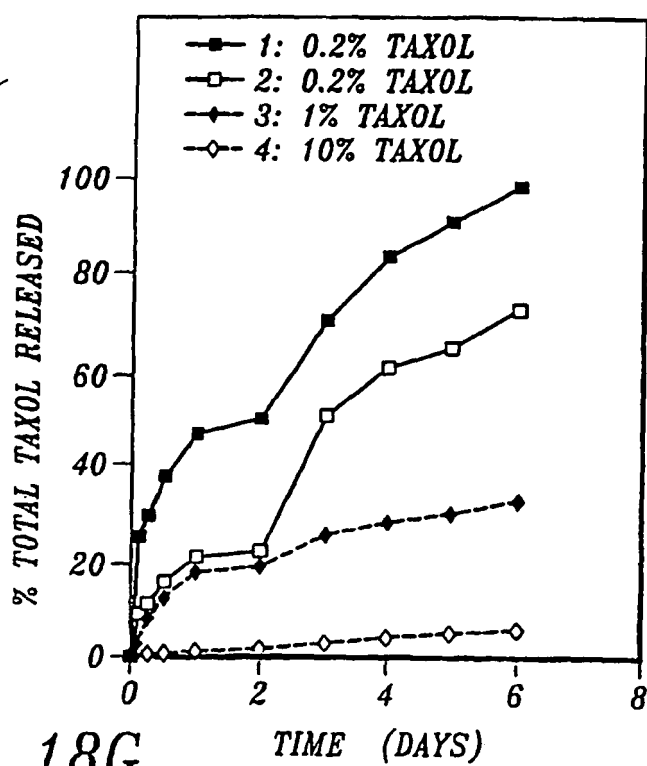
FIG. 18G.

FIG. 18H.

FIG. 19A

FIG. 19B

FIG. 20A
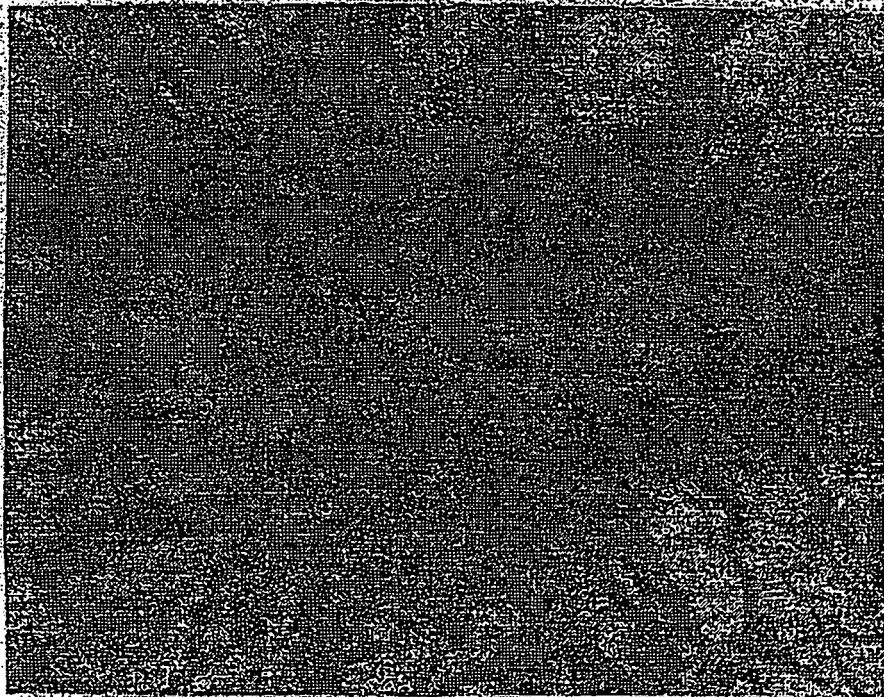
FIG. 20B

FIG. 20C

FIG. 20D

FIG. 21A.

Control   20% taxol-paste
cross sections of tumors

FIG. 21B

20% taxol-paste   10% taxol-paste
cross sections of tumors
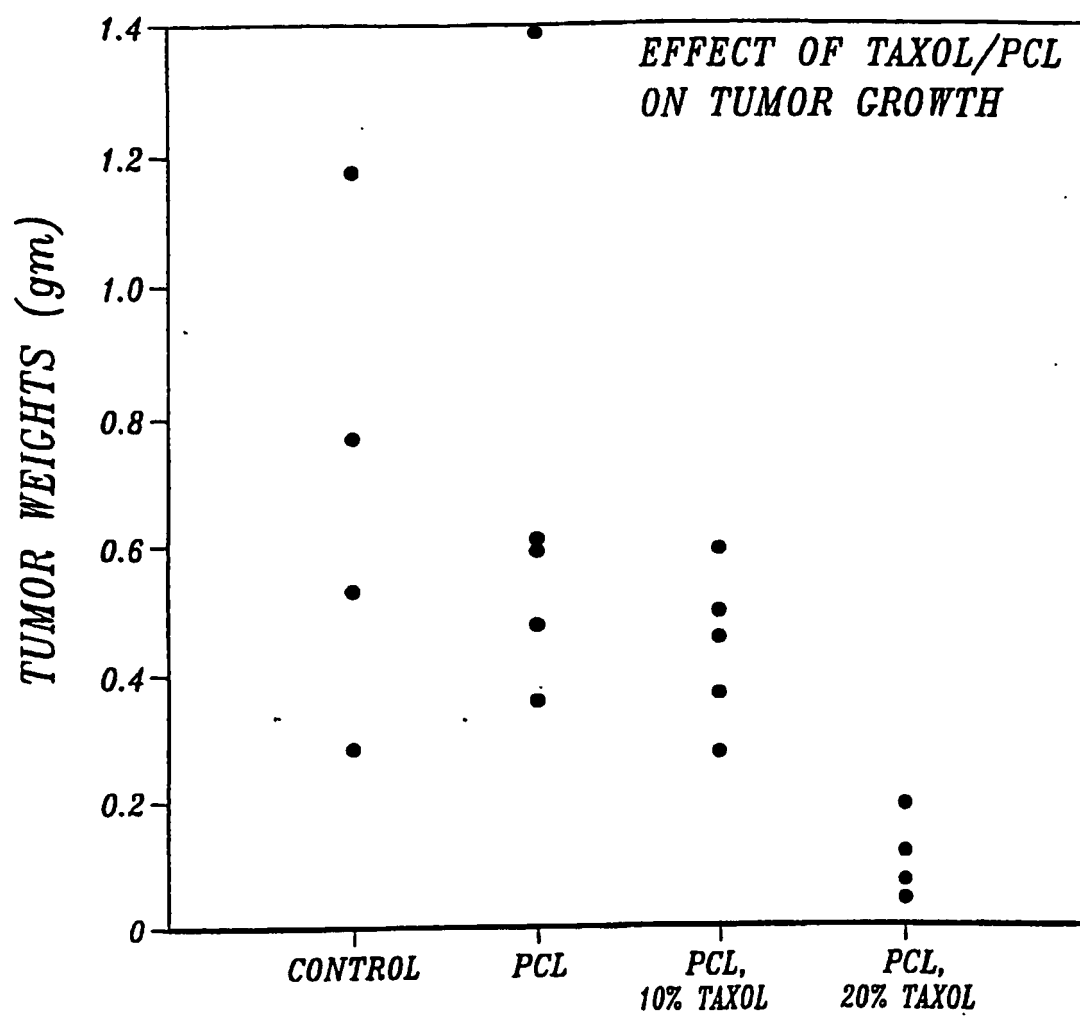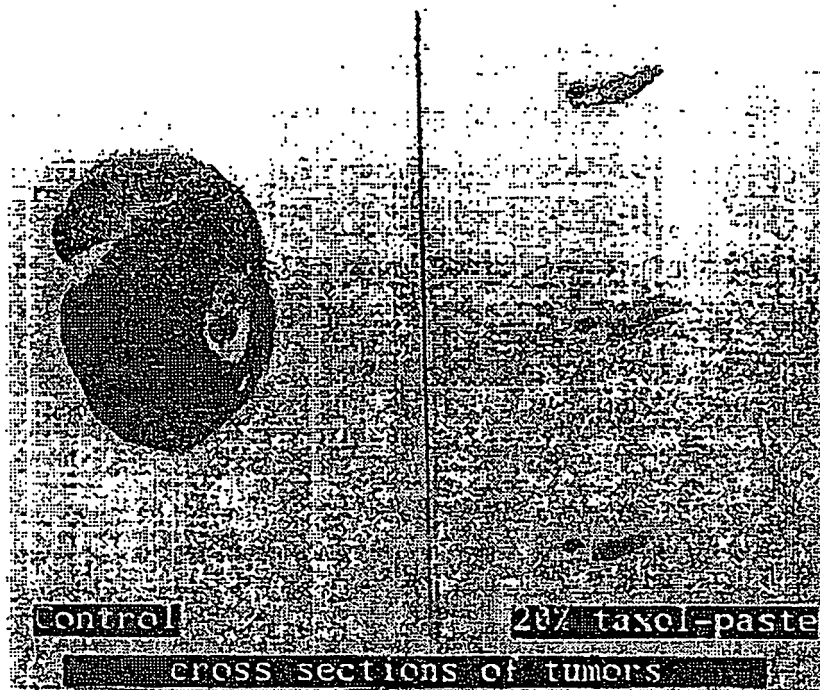
FIG. 21C

REFERENCES CITED IN THE DESCRIPTION

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